D. KINGSLEY

Revenge of the hopeful monster

Experiments have revealed how single mutations can have huge effects that drive evolution. But small steps pave the way, finds **Tanguy Chouard**.

Since the origin of evolutionary science, biologists have insisted that adaptation is an achingly slow process. '*Natura non facit saltum*' (nature does not take leaps) was a favourite incantation of Charles Darwin. As the combined power of genetic mutation and natural selection became better appreciated in the 1930s and 1940s, theorists solidified a gradualist doctrine: adaptation must rely on innumerable genetic changes, each with effects so small that any attempt to catch them experimentally was considered futile.

Suggestions to the contrary were met with ridicule: geneticist Richard Goldschmidt, in 1940, envisioned subtle developmental mechanisms producing great leaps of adaptation, but his use of the phrase "hopeful monsters" was misrepresented as extreme saltationism (perfection in one jump), and equated with belief in miracles. But through fish in the murky depths of a British Columbia lake and through bacteria in the flasks of a Michigan lab, the monsters have returned. Experimental evidence has shown that individual genetic changes can have vast effects on an organism without dooming it to the evolutionary rubbish heap.

Single-gene changes that confer a large adaptive value do happen: they are not rare, they are not doomed and, when competing with small-effect mutations, they tend to win. But small-effect mutations still matter — a lot. They provide essential fine-tuning and sometimes pave the way for explosive evolution to follow. As the molecular details unfold, theory badly needs to catch up.

Mice without the gene died. That didn't look like a promising way to evolve new traits.

"This is a very exciting age," says Joe Thornton, who studies protein evolution at the University of Oregon in Eugene: new molecular approaches are bringing mechanistic understanding to the field of 'evo-devo' (evolutionary developmental biology), ushering in what Thornton calls "the functional synthesis"[1]. The shift even promises to bridge microevolution and macroevolution, suggesting how, for example, genetic changes — large and small — might eventually lead to a new species.

Marine gladiators

The promise of such insights was what drew developmental biologist David Kingsley from Stanford University in California and then-postdoc Katie Peichel, to the three-spined stickleback (*Gasterosteus aculeatus*). To move beyond evo-devo studies that compared gene expression differences between vertebrate species, they decided to identify the genetic changes that actually caused variation in body plans. Sticklebacks were chosen because populations in different environments can look very different but can produce fertile hybrids through *in vitro* fertilization (IVF). So, in 1998, Kingsley and Peichel flew to Vancouver, Canada, to start a collaboration with Dolph Schluter, an ecologist and evolutionary biologist at the University of British Columbia, and an expert in stickleback IVF.

They used two stickleback populations: a heavily armoured one, from a patch of sea north of Vancouver, that sports a pair of sharp pelvic spines, and one that lives at the bottom of British Columbia's Lake Paxton and is relatively naked. With Schluter's help, one saltwater female and one freshwater male produced a slew of grandchildren with features anywhere between gladiator and nudist. In particular, they exhibited pelvic spines of all sizes. Such features that vary along a continuum are known as quantitative traits and can be linked back to large segments of genomic DNA — the quantitative trait loci (QTLs) that cause them. By analysing the DNA of hundreds of hybrid fish, the Kingsley group spotted one particular QTL in the genome responsible for two-thirds of all variation in pelvic spine length[2].

Such large-effect QTLs were not unheard of — many had been shown to vastly change the sizes of leaves or fruits in domesticated plants,

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A short segment of engineered DNA brings back the pelvic spines lost in stickleback evolution.

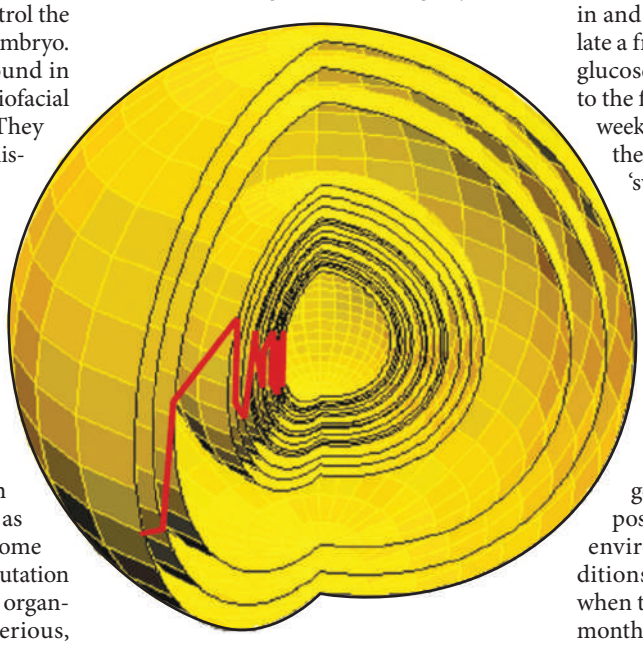
for example — but the reigning gradualist dogma regarded these as artificially protected monstrosities that would never survive the harsh hand of natural selection. It remained unclear whether plant QTLs found in natural populations corresponded to individual genes. To map vertebrate QTLs at higher resolution, Kingsley needed greater statistical power. "The combination of Dolph's large hatcheries with our dense physical maps of the stickleback genome allowed us to zero in on individual genes quite effectively," says Kingsley. In 2004 they found one gene[3], *Pitx1*, but it had a problem: it seemed to control the development of too many parts of the embryo. When disrupted, the gene, widely found in vertebrates, left mice with severe craniofacial abnormalities and pituitary defects. They died at birth. "That didn't look too promising to evolve new traits," says Kingsley.

Pleiotropy, the way in which a single gene can affect many factors, had been a major rationale behind gradualist dogma. In the 1930s, theorist Ronald Fisher formalized the intuition that mutations with large effects should be deleterious[4]. He conceived of an organism's quantitative traits as the axes of a multi-dimensional space with optimal fitness at the centre. In this model (see figure, right), an organism maladapted to its environment, such as a spiny stickleback in freshwater, lies some distance away from the optimum. A mutation may either be beneficial, nudging that organism closer to the optimum, or deleterious, pushing it further away. According to Fisher's model, pleiotropic mutations, such as the deletion of *Pitx1*, reduce the spine size, but push other traits fatally away from optimal fitness. How could a mutation in such a crucial gene result in anything but a hopeless monster?

Further inspection of the freshwater stickleback *Pitx1* gene revealed a DNA lesion far more subtle than the gene knockout in mice: it removed some 500 base pairs of regulatory sequence that enhances Pitx1 protein production only in the pelvis[5]. With expression of the Pitx1 protein preserved in all other vital structures, freshwater sticklebacks could lose their pelvic spines without dire repercussions elsewhere. "This made so much sense," says Kingsley. Thanks to the way regulatory DNA sequences are organized — as independent modules — evolution had managed a surgical strike on *Pitx1*, resulting in a beast hopeful and not so monstrous after all.

This event is far from rare: freshwater sticklebacks with reduced pelvic structures fished from various sites across the world have independent deletions in the same DNA region, all more or less eliminating the marine pelvic enhancer. But how could such an improbable surgical strike happen over and over again just by chance? Recently, Kingsley's team showed that four of the ten stickleback DNA segments predicted to be most susceptible to deletion are in *Pitx1*, one right next to the pelvic enhancer[5].

The work, building on plant studies, showed that single-gene lesions could produce large morphological effects and yet be viable in natural situations. Such physical effects also seemed advantageous, but Kingsley's team could not prove this. This is because QTL studies map genotype (the DNA sequence) to phenotype (observable traits) but do not measure the resulting changes in fitness — actual reproductive success. To measure fitness, researchers need to watch mutants as they compete in real time. And the easiest place to watch this race as it happens is in the lab.



In this representation[4] of Ronald Fisher's model of adaptation, beneficial mutations can slowly bring a population (red line) closer to the optimum at the centre, but most large-effect mutations will be deleterious.

Catching evolution red-handed

More than 20 years ago, Rich Lenski, then at University of California, Irvine, started a simple experiment. On a Wednesday morning, the 24 February 1988, he started the parallel evolution of 12 populations of *Escherichia coli*, all clonally derived from a single bacterium and competing for limited sugar in Erlenmeyer flasks. Each day, roughly half a billion new copies of the *E. coli* genome are made in each flask as the bacteria multiply, along with about a million mistakes, meaning that in the span of a few days, virtually all conceivable mutations in the bacteria's five million base pair genome will have been attempted. Most of these mutations make no difference or are deleterious, but a few make the bacteria grow a little bit faster — providing up to a 10% growth advantage over their predecessors. The fastest ones are extremely rare and the population must 'wait' a couple of days for them to show up.

Every night, the bacteria run out of the sugar glucose and go dormant. The following day around noon, a researcher plunges a pipette in and sucks up 1% of the culture to inoculate a fresh flask. Those faster at gobbling up glucose will send more of their descendants to the following day's pipette and, after a few weeks, descendants of the fastest one will be the only ones transferred as the mutation 'sweeps' to fixation.

To draw a parallel with an Aesop's fable, a small-benefit mutation, with its slow sweep but short wait, would be the tortoise of this race, whereas a large-benefit mutation, with its fast sweep but long wait, would be the hare. But in real life, whether wait or sweep matters more will depend on many factors, such as mutation rates (which vary from gene to gene), genome size, population size and composition (which both vary over time) and environmental conditions. Lenski's conditions seemed to favour the hare, at least when the researchers first looked. Just a few months in, Lenski and his students observed two striking things: the evolving bacteria's relative fitness — their growth advantage over the ancestor — increased in abrupt jumps. And these jumps became smaller in later generations (see graph, overleaf). The jumps

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seemed to correspond to individual mutations taking over the population, one after the other: large-benefit mutations seemed to respond to the initial, abrupt environmental change, followed by smaller ones for 'fine-tuning', if allowed enough time and stability[6].

This fits well with Fisher's model. With each step towards the centre, the sphere representing an organism's adaptive space shrinks, limiting the potential pay-off from the next mutation[4].

There are exceptions to this 'large-early, small-late' rule more recently gathered by the Lenski team. First, some of the earliest mutations provided minuscule fitness gains. Second, some of the largest-effect mutations took tens of thousands of generations to become fixed.

One of the earliest mutations to get fixed (less than 2,000 generations), and in all 12 populations, was the deletion of the 'ribose operon', a cluster of genes used in breaking down the sugar ribose. Getting rid of it provides a sluggish (1–2%) selective advantage against the ancestor on a glucose-only diet[7]. But there's a reason it gets fixed so early. The ribose operon is a favourite destination for transposons (bits of DNA that cut, copy and paste themselves throughout the genome), and one in every 50 mutations popping up wipes it out. Even though such mutations provide only a tiny advantage, they will almost certainly sweep to fixation. It would take a very fast hare to catch up with an army of early tortoises such as these.

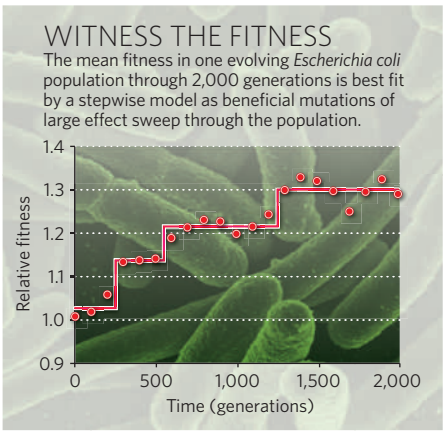
Gaining the ability to use citrate must have been like the colonization of land by proto-tetrapods.

The rise of *Escherichia erlenmeyeri*

Another type of rule break has proved to be more surprising. One morning, at the turn of generation 33,127 according to the lab's log book, a massive increase in turbidity was recorded on the vial labelled Ara-3. The sugar-starved bacteria had suddenly 'discovered' a vast new source of carbon by importing citrate, a pH buffer that had been in the growth media all along, and it sent the population size through the roof. This Cit+ phenotype happened in only one of the twelve populations and took more than a decade to show up[8]. It has been compared to the evolution of a new species, jokingly christened *Escherichia erlenmeyeri*. "It must have been like the colonization of land by proto-tetrapods," says Lenski, now at Michigan State University in East Lansing. 'Land' (citrate) had been there for quite a while, but it was only after that first breakthrough that new horizons were opened and further changes could evolve. In a Fisher model, this would amount to teleportation to a new adaptive sphere.

To find out the mechanism behind the exceptional and extremely slow evolution of the Cit+ innovation, Lenski and his colleagues went back to their frozen fossil record: *E. coli* samples they had been periodically storing in a –80 °C freezer. They then attempted to replay the evolutionary tape. They revived hundreds of specimens, made hundreds of replicate cultures of each and pushed them through thousands more generations. They ended up testing close to 50 trillion individual cells for the ability to grow on citrate without glucose, but cornered only a handful of 're-evolved' Cit+ mutants. All were derived from recent samples. The capacity to exploit citrate could not evolve from the ancestral *E. coli* genome in one step. Instead it required a minimum of three mutation events[8]. Smaller mutations had to set the stage for the later, more dramatic event. A given mutation often seems to depend on modifier mutations — a phenomenon known as epistasis — and thus on the organism's history before it can have a large effect.

It remains to be seen whether such elementary mechanisms of adaptation, often referred to as microevolution, can instruct the higher processes that constitute macroevolution, such as speciation and the emergence of biodiversity or complex organs. In 1996, Lenski and his colleagues noted that the adaptive jumps in his bacterium's cell size were reminiscent of the abrupt morphological changes punctuating long periods of stasis in the palaeontological record[9] publicized 24 years earlier by palaeontologists Stephen Jay Gould and Niles Eldredge. Lenski meant to show how such seemingly complex punctuated equilibria could arise by the simplest — and indeed most orthodox — mechanisms in Darwinian population genetics: mutation and natural selection (wait and sweep). At that time, some evolutionary biologists



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dismissed the case as irrelevant to Gould and Eldredge's much-disputed theory because, they said, there was neither speciation nor species selection in Lenski's studies. But that was years before the 'new land' of citrate was discovered in vial Ara-3.

Ara-3 now essentially had biodiversity. As the new Cit+ clique gorged itself with citrate, it became less interested in glucose, leaving space for a 1% minority of Cit– glucose experts, which now discretely coexist in the same vial. This is remarkable, Lenski says, because the total absence of sexual reproduction, or any form of horizontal DNA transfer, in his experiment normally leads to a no-prisoners-taken mode of evolution, in which the best genome of the moment forces all others to extinction — a phenomenon called clonal interference. From the moment they stop competing for the same resources, however, species can coexist, a principle thought to force the tree of life to fan out[10]. "From so simple a beginning," as Darwin envisaged, a single-cell ancestor and its descendants gave rise to their own, microbial version of his beloved "entangled bank".

Jerry Coyne, an evolutionary biologist at the University of Chicago, Illinois, urges caution. "Nobody agrees about what speciation means in bacteria," he says, and although the concept of adaptation is similar in bacteria and sexual organisms, speciation is a different ball game. Lenski argues that the emergence of the Cit+ variety is about as close as it gets to speciation particularly as the inability to use citrate in the presence of oxygen is a defining feature of natural varieties of *E. coli*.

But he also emphasizes at least two properties that make it difficult to generalize the dynamics of his bacterium's evolution to more

SOURCE: R. E. LENSKI *PLANT BREEDING REV.* **24**, 225–265 (2004)

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After more than 33,000 generations of evolution, one bacterial population (cloudy flask) exploded in growth.

complex organisms: bacteria have relatively high DNA mutation rates and the particular strain he uses is fully asexual.

Of form, function and fitness

More complex organisms such as sticklebacks present extremely low rates of new mutation — with the remarkable exception of the mutation-susceptible *Pitx1* gene — and so must rely on other mechanisms to produce genetic variation. Some of Kingsley's work illustrates this. Besides the shrinking of pelvic spines, his group has identified individual mutations that have large effects in two more morphological traits that get reduced in freshwater fish: the number of armour plates is chiefly controlled by ectodysplasin (EDA) and skin pigmentation by kit-ligand (KITLG), both key developmental signalling proteins. In each case, the researchers found that a copy of the freshwater 'version', or allele, of the gene is carried by a small percentage of marine sticklebacks. Those carrying it often have an intermediate phenotype. This standing variation is probably maintained by regular mixing of marine sticklebacks with those in coastal streams[11,12].

Each time a population moves between sea and lake, the selective pressure shifts and the allele frequencies respond, with major morphological consequences. This, the researchers found, has happened independently many times, and the very same alleles have been seen to swap throughout the Northern Hemisphere. As marine sticklebacks populated lakes at the end of the last ice age, 10,000–20,000 years ago, Kingsley considers this part of a very fast speciation event. But changes at a single QTL don't have to be a single mutation. For both the *Eda* and *Kitlg* genes, the freshwater and marine alleles differ at hundreds of scattered positions. The researchers don't yet know which ones control phenotype. They do know, however, that most of the DNA changes accumulated over the millions of years since sticklebacks first migrated between salt and fresh water. Individual QTLs that may provide large and immediate benefits in the short term when environmental conditions change, could thus have evolved previously — through the accumulation of myriad mutations of small effects over immense periods of time. To study those mutations, Kingsley is collaborating with scientists at the Broad Institute in Cambridge, Massachusetts, to sequence full stickleback genomes from ten marine and ten freshwater individuals collected around the world.

"Nobody agrees about what speciation means in bacteria."

Large effect or small, evolution begins to look like an endless list of special cases, each a new challenge to Fisherian models. One reason is the general lack of knowledge about how changes in genes contribute to function and how this affects fitness. "Current population-genetics models consider the molecular details of how mutations control phenotype as statistical noise," says Paul Rainey, an evolutionary microbiologist at Massey University in Auckland, New Zealand. This is what Thornton hopes will be corrected by a "functional synthesis", marrying evolutionary biology, molecular genetics and structural biology. Thornton and his colleagues have begun to look at the evolutionary constraints placed on proteins. They have studied a regulatory protein — the glucocorticoid receptor — as the protein evolved from one species of fish to another over millions of years. They combined X-ray crystallography and biochemistry to dissect how the protein's structure constrains its stability and function, namely which hormones it can bind. They found that key function-switching mutations (which swap amino acids directly in contact with the hormone) depend on smaller-effect mutations at distant positions, to determine which route adaptation can actually follow under natural selection — a mechanism they call an 'epistatic ratchet'[13].

Many researchers have welcomed the return to favour of large-effect mutations and have resurrected Goldschmidt's long reviled idea of the hopeful monster. But they can't ignore the small-effect mutations. "We need much more data before the issue of large versus small can be settled", says Coyne. Kingsley, like Coyne favours a middle-ground view, in which neither large- nor small-effect mutations are ruled out. "Our work has too often been portrayed as saying that Darwin was wrong" about big leaps in adaptation, he says. But in fact, none of the traits his group has studied is completely due to the effects of a single gene. *Pitx1* accounts for only two-thirds of the variation in pelvic-spine length. Moreover, Kingsley says, "We've got more than 150 other QTLs to study."

The difference between large and small effect mutations can be sliced many ways, and it greatly depends on context. A mutation may affect phenotype but not change fitness much. It may have a large effect in the context of a given genome, or in a given environment, but may have a smaller effect later in an organism's history. As researchers drill down to the molecular mechanisms driving adaptation, theory may catch up and dogmas may recede. Ultimately, says Kingsley, "we should let the bacteria, the fish and other organisms speak". ■

Tanguy Chouard is a senior editor for *Nature*.

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