Co-opting a Blind Watchmaker

Biochemists are putting evolution to work in the test tube, hoping to produce new catalysts and drugs—and gain insights along the way into evolution in nature

To the biologist Richard Dawkins, evolution is a "blind watchmaker," able to produce structures of marvelous intricacy through a series of accidents. Now some biochemists and biotechnologists are trying their hand at the same game, hoping for some of the same luck. In search of useful new molecules, they are putting the basic principles of Darwinian evolution—chance variations followed by selection—to work in the test tube.

Traditionally, researchers searching for a new drug or industrial chemical have had to rely on the painstaking process of molecular design, which restricts them to only those molecular structures they can envision. Or they have had to sort through the molecules already available in nature. But evolving molecules in a test tube liberates chemists from the limits of nature, and from the limits of their own imagination as well. "That's why these evolutionary technologies are a great advantage—you can solve problems in ways you never would think of," says Manfred Eigen, a Nobel Prize–winning chemist at the Max Planck Institute for Biophysical Chemistry who is also chief scientist at Evotech, a Hamburg-based start-up company.

The basic idea, says chemist Gerald Joyce of the Scripps Research Institute in La Jolla, is to mutate a starting molecule to produce millions or billions of variants. Then apply some kind of selection process to filter out the "fittest" variants—those that outdo their fellows in, say, catalyzing a reaction or binding to a pathogen. Finally, take the winner molecules, add new mutations, and apply the selection pressure once again. Repeat the process over several generations, says Joyce, "and you have full-blown evolution." The end result can be molecules with entirely new properties created, in effect, by accident.

The field is just starting to blossom, say researchers, but already it promises to deliver new enzymes for food processing and detergents, as well as enzymes that can withstand heat or solvents poisonous to most biomolecules, making them viable replacements for the less precise inorganic catalysts used in industry. It has also yielded a harvest of basic research, including new insight into how RNA may have developed the ability to replicate itself, a step many researchers think was critical to the evolution of life.

For biochemists accustomed to carefully tailoring molecules, the power of chance variation can come as a revelation, says Gregory Petsko of Brandeis University. A few years ago, he recalls, he and colleagues were tinkering with a bacterium's genetic machinery to try to increase its output of a particular protein. "We went to a lot of trouble," he says, but still did not get very good yields. But then a chance event in just one cell out of the millions happened to give them just what they wanted. The bacterium "took our gene and moved it thousands of bases away from where we had it," Petsko says. "The bug just did that by itself ...it was totally random."

Evolution on fast-forward

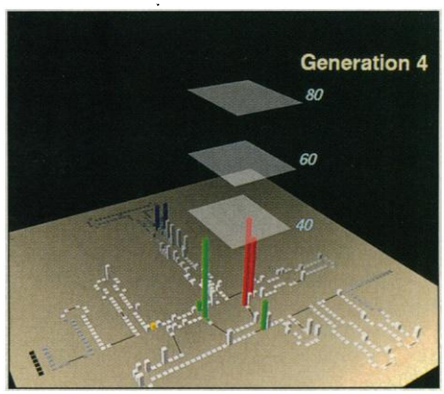
Since then, Petsko has built on this insight by splicing genes for various enzymes into fast-breeding strains of *Escherichia coli* and using evolution to change the enzymes' functions. Petsko and his colleagues get the mutations that are the raw material of evolution naturally by using bacterial strains that are prone to making mistakes when they copy their DNA. Or they do it themselves by making copies of the gene with an intentionally "sloppy" version of the polymerase chain reaction (PCR), the popular technique for reproducing nucleic acids, and then reintroducing it into the bacteria.

To create the selection pressure, they expose the bacteria to conditions that favor the desired enzyme function—for example, the ability to convert the sugar xylose to xylulose, a step important in the manufacture of some sweeteners. By growing xylulose-loving bacteria on a medium rich in xylose, Petsko and his colleagues evolved an enzyme that can make the conversion. And they are putting the same principle to work to alter other enzymes as well.

Frances Arnold, a biochemist at Caltech, is also working with bacterial enzymes, but she is trying to coax their evolution in a different direction—toward the ability to function in organic solvents. Because such solvents never turn up in our water-based world, says Arnold, "You are looking for an enzyme that nature never had a reason to make." But technology now supplies a reason: Such an enzyme would be valuable in the large-scale manufacture of many chemicals and pharmaceuticals, she says.

And evolution, it seems, can answer the need. Last year Arnold evolved an enzyme that works in a solvent called dimethylformamide (DMF). She started with a batch of *E. coli* that had been genetically engineered to make the original enzyme, called subtilisin. Subtilisin breaks up chains of amino acids, an ability that could be valuable in the synthesis of some drugs. Natural subtilisin, however, is crippled by the solvents often used in drug manufacture. But by subjecting the bacteria to solutions increasingly rich in DMF, Arnold was able to identify a few mutant individuals whose enzymes still retained a hint of function. She then isolated the subtilisin gene from these bacteria and copied and mutated it by sloppy PCR. After splicing the copies back into a new batch of bacteria, she subjected this new generation to a higher dose of DMF. By repeating this procedure over just three generations, Arnold developed an enzyme that works more than 200 times better in DMF than natural subtilisin.

By artificially generating mutations, Arnold and Petsko put evolution on fast-forward in their bacteria. But Willem Stemmer of the biotech firm Affymax, in Palo Alto, says he has a way to speed up the process even more—through a test-tube version of sex. What Stemmer is mimicking is the gene shuffling that takes place during the formation of sperm and eggs. In this process, two different versions of each gene pair up and swap parts, sometimes producing improved genes that are passed on to the offspring. In Stemmer's in vitro version, bacterial genes are artificially reshuffled to produce favorable new combinations of traits.



Evolution pinpointed. The frequency of mutations at sites on a catalytic RNA changes as successive generations of molecules are subjected to selection for the ability to function under new conditions.

Stemmer demonstrated this strategy by evolving a strain of *E. coli* that resists the

antibiotic cefotaxime. He started with colonies carrying a gene for β-lactamase, an enzyme that breaks down some antibiotics but is largely ineffective against cefotaxime. After exposing a first generation of bacteria to the drug, he selected out a few survivors. But instead of simply copying and mutating the survivors' genes with sloppy PCR, Stemmer first chopped up the genes into random fragments. He then used a PCR variant to stitch gene fragments from as many as 10 different individuals into a new set of genes, which could be reintroduced into bacteria and undergo another round of selection.

Shuffling the deck this way gives a few individuals a big advantage, Stemmer explains. The β-lactamase genes in the first generation of survivors probably contained just one beneficial mutation each, but by taking them apart and recombining them, he gave a few individuals a winning hand—a whole array of beneficial mutations. The results bore out this promise, he says. After just three generations of sexual PCR and selection, the *E. coli* showed a 16,000-fold increase in resistance to cefotaxime. Bacteria evolved using ordinary sloppy PCR, in contrast, showed a mere 16-fold improvement.

Reshuffling the deck is one way to better the odds of quickly finding beneficial mutants. Another way to jack up the odds is to enlist larger populations of organisms or molecules, containing more mutational variants. With bacteria you are limited to working with few million cells at a time, notes Joyce. Use smaller, simpler viruses as the vehicles for your evolving molecules, though, and each generation can number in the billions. That is the approach being taken at Evotech, which is enlisting the bacteria-infecting viruses called phages to evolve new enzymes and to do basic research on rapid viral evolution—the phenomenon that makes the AIDS virus so hard to fight.

Other groups do away with organisms altogether, practicing a stripped-down version of test-tube evolution in which they rely only on the tools of biochemistry. Take Joyce, who is practicing evolution on bare molecules of RNA. Selection—natural or artificial—generally acts on proteins, the doers of the biochemical world. But Joyce's RNA molecules, known as ribozymes, not only carry genetic information but behave as enzymes as well, since they can catalyze chemical reactions. Thus the same set of molecules can be mutated and replicated—and then selected and mutated again to alter the ways they perform their jobs as enzymes.

Autonomous molecules

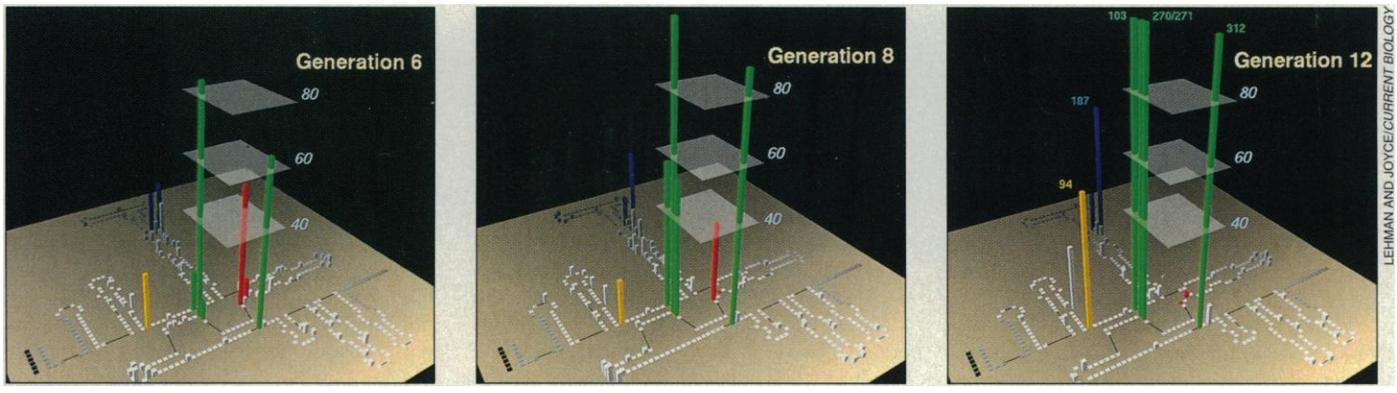
Joyce begins by assembling random sequences of nucleotides to generate a vast "library" of RNAs. He then sorts through it for the few variants that do the best job of binding to a given target or catalyzing a given reaction, amplifies and mutates those variants, and then repeats the enzymatic test. Two years ago, by continuing the process over 10 generations, Joyce succeeded in evolving ribozymes with a new catalytic ability. In nature, ribozymes can cut and splice their own RNA, but Joyce's altered ribozymes could cut DNA instead (*Science*, 31 July 1992, p. 635).

Since then, he says, he has been trying to develop a test-tube RNA with a more startling trait: a life-like ability to replicate and evolve without any outside help. His RNA molecules have already taken one step toward this kind of autonomy. They have evolved the ability to replicate without primers—short stretches of matching RNA normally needed to start the action of the copying enzyme. He and his colleagues started referring to this newly independent RNA as "the beast." On close examination, the beast seemed to have succeeded in evolving its own embedded promoters—regulatory sequences that signal for replication to begin.

Harvard biochemist Jack Szostak, who also works with ribozymes, has taken a different step toward a self-replicating molecule. He says he recently succeeded in evolving a strand of RNA that could piece together a complete copy of itself by joining other RNA segments, each of which contains part of the original RNA's nucleotide sequence. That is still several steps away from an RNA that could copy itself from scratch, but he thinks that goal is within reach. "Whether you accept this as meeting the definition of life is up to you," says Szostak, but if you gave such a self-replicating molecule an envelope of fat or protein, he says, "it would look a lot like the first cells that arose on Earth."

One Seattle-based startup company, fittingly named Darwin, is developing a version of in vitro evolution that looks much less like life, because its medium is not biomolecules—proteins or nucleic acids—but simple molecules like small aromatics and amino acids that are the basis of many drugs. David Galas, Darwin's chief scientist, explains that because small molecules are not the direct products of genes, it is much harder to mutate them or copy them in quantity. "That's why small molecule evolution is a more substantive technical challenge," he says.

So far, Darwin has mastered only the mutation step. By starting with a molecule that has 10 reaction sites with 10 potential attachment groups at each one, for example, Galas and his colleagues can spin out as many as 10^{10} variants. They can then select over that one generation for, say, the ability to aid or hinder blood clotting or bind to some receptor involved in a disease. That is not so different from a technique known as combinatorial chemistry, now widely used for drug development (*Science*, 3 June, p. 1399). But Galas and his colleagues are now hoping for a way to amplify selected molecules and repeat the process, enlisting the full power of evolution. In small-molecule evolution, as in the field as a whole, "the really clever things are yet to be invented," says Galas. "It's a wide open field."



–Faye Flam

Additional Reading

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