The one-way road of Müller's ratchet: a way of understanding how mutations build up in an organism

The genesis of DNA

Mathematical model describes the competition between molecules that made it possible for living beings to emerge on Earth

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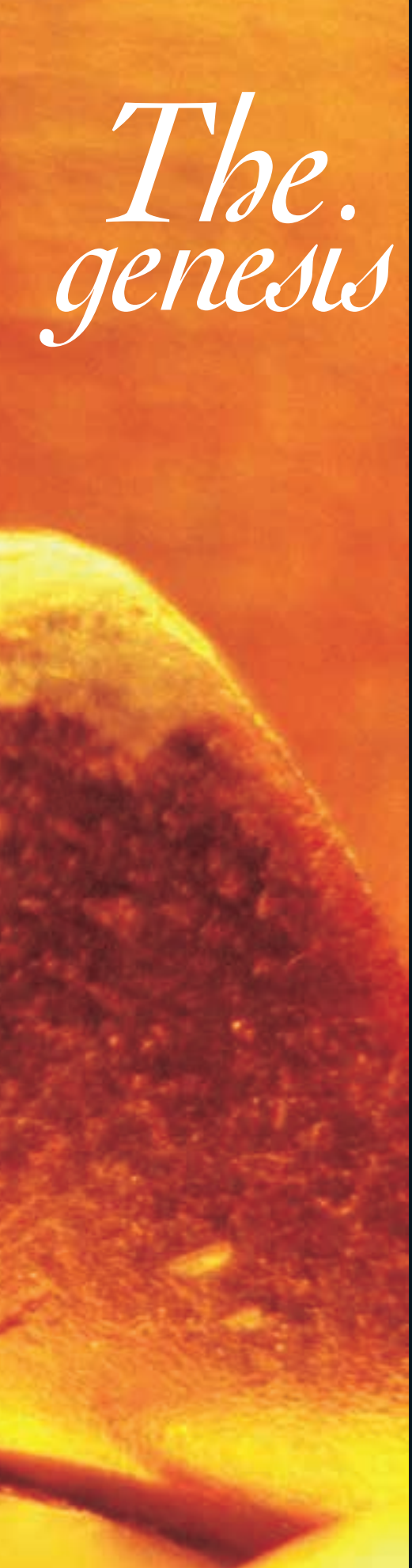

PHOTOGRAPH BY MIGUEL BOYAYAN

An icon of modern science, the DNA molecule is the sole survivor of a struggle that lasted millions of years. Unmistakable for its two intertwined strands, it emerged from an intense competition with other chemical structures that were capable of copying themselves. It only triumphed because there was cooperation, or, to borrow a term from anthropology, altruism, amongst them. The more refined molecules, which managed to buy time copying themselves by means of enzymes, a kind of protein, helped the more primitive ones, probably in an involuntary way, which would generate replicas of themselves by more lengthy methods. The entirely selfish ones, for some reason unable to offer help, simply disappeared. Only after the conclusion of the process of selection amongst the participants that were getting fitter all the time was it that the first organisms started to form themselves on Earth, a probable 4.5 billion years ago.

The reconstitution of the backstage of life on the planet with this new ingredient, cooperation between molecules, results from work carried out not by a chemist or biologist, as one might expect, but by a physicist from Rio Grande do Sul, José Fernando Fontanari, from the São Carlos Physics Institute (IFSC) of the University of São Paulo (USP). Seven years ago, he suspected that the formulas that he used to present to his students in statistical mechanics did not serve just to describe the ways by which atoms react to one another. If looked at as a model for interaction amongst particles, it would perhaps solve wider problems, which biologists dealt with only in a conceptual fashion, perhaps for feeling ill at ease with equations and mathematical models.

His hunch was right. In two recent articles, published in October and November 2002 in *Physical Review Letters*, Fontanari describes mathematically how the selection process took place that led to a single victorious molecule, DNA, whose elegant structure was discovered exactly 50 years ago, as a result of a joint work between physicists and biologists, and which is so well known today that there is no longer any need for reminding that this is the acronym for deoxyribonucleic acid.

By bringing physics close to biology, Fontanari solved a few paradoxes that had been aired 30 years ago by German chemist Manfred Eigen (1967 Nobel prize for Chemistry). Eigen had created

the theory of replicators, molecules that succeed in making copies of themselves, and today, in their more refined version, DNA, store information that starts the process for producing proteins, indispensable for the formation of all the parts of living beings. Capable of attracting smaller fragments that, joined together, would result in a copy of itself, the first replicator arose by chance. "It was an accident of history", says Fontanari. But it was enough to change the pattern for producing molecules, before that formed by simple aggregation of blocks, as if they were Lego pieces joining themselves together at random.

In the beginning, enzymes would help selfish molecules

Were it to depend on this first replicator, life would have no future on Earth. For being so small, it could not store sufficient information to start making proteins. It managed to copy itself by acting as a mold for itself, but the process was still too slow and prone to errors, which would become more and more frequent as it grew. "The larger the molecule, the more difficult and time-consuming it is for it to make a copy of itself", says the physicist, supported by proofs in experiments. "The probability of the first replicator making a perfect copy of itself was practically nil."

Strategic leap - There was another historical accident some time afterwards. Inaugurating the third pattern for making molecules, which persists until today, the descendants of the first replicator, now different from the original because of the accrued mistakes, manage to create intermediary molds – here are the enzymes, a kind of protein that speeds up chemical reactions. With them, the replicator gains time, avoids mistakes, and generates more copies of itself. It is also more protected from the attacks of other molecules, a situation close to the one found in some kinds of virus, in which a molecule acts as a cover for the genetic material.

When formulating this thesis, Eigen noted that there was something odd, which was to be known later as the paradox of altruism. By creating an enzyme, instead of simply carrying on copying itself, the mutant molecule, which starts this new generation of replicators, does something that would not only be used by itself, but would also benefit the replicators that were still copying themselves by a mold. "Eigen solved the problems of the chemical complexity of the origin of life, but he didn't realize that this would mean there was altruism among the first more evolved molecules of Earth", Fontanari comments. English biologist John Maynard Smith, from the University of Sussex, England, repudiated this idea for thinking that it was impossible for there to be altruism amongst molecules.

Examining the impasse, Fontanari concluded that this new replicator had to pay a price for this new skill: it could not copy itself while it was creating the enzyme. It is the same situation met by a worker who earns according to the number of bottle tops that he can fit by hand. He can put the caps on more quickly by building a machine, but while he is building it he fails to meet the production target and earns less than his companions, for whom manual work is inevitable.

Compared with the selfish molecules, which had not stopped spawning copies of themselves nor did the replicating molecule see itself at a disadvantage and therefore ran the risk of extinction. It would only fail to be in a pickle if the enzyme would only work for itself – something improbable in the biochemistry of those times. In this way, the protein is going to assist other replicators, which use its advantages at no cost to themselves.

Isolation and mixture - An impasse emerged, however. "The mathematical study of the dynamics and the evolution of these two kinds of replicators competing for their building blocks shows that the enzymatic replicators can neither invade nor coexist with the population of mold-type replicators", the physicist comments. "But we know that an invasion must have happened, since the current replicators are of the enzymatic kind." How to get out of this and explain altruism, an apparent disadvantage? Fontanari solved the riddle by showing mathematically that the enzymatic replicator manages to survive, even being generous with its companions and lending them its precious enzyme, provided that it is confined to a limited space or can not move much, so that the enzyme remains close to the mother molecule.

The equations agree with a hypothesis that enjoys growing acceptance among biologists, according to which life arose in cracks in rocks, particles of mud, or drops of water, which would favor the confinement of the molecules. Another point that reinforced the thesis is that there is now no more talk that the first replicators emerged in a mixture, the primordial soup, but in a flat space, similar to a pizza – something like the surface of pyrite, an iron oxide based mineral, the best bet for housing the ancient forms of life. Passing from a space of three to one or two dimensions, the chemical reactions would take place more easily.

That was still not enough. If they remained isolated, the enzymatic replicators, being altruists, would be eliminated by the others, the selfish ones. For this reason, Fontanari argues that, besides confinement, there has to be a mixture between the groups of mole-

PHOTOGRAPH BY MIGUEL BOYAYAN

Pyrites, on which life may have arisen: pizza instead of soup

cules. "Due to the tides or to the wind, the group are periodically mixed together and are redistributed in a random manner in the compartments", he says. "In this redistribution, clones of the enzymatic replicators, more numerous for succeeding in copying themselves more quickly, have better chances of going back to the compartments, while those that do not succeed literally go with the wind." This is the moment at the enzymatic replicators recover from their initial disadvantage, since the mixture makes it possible for the selfish ones to leave groups that are abundant in enzymes due to the presence of the altruists. When they fall into groups that are poor in enzymes, the selfish ones lose the power of replication and allow the altruists to become isolated. "Mathematically, it is shown that a repetition of this process ends up leading to the predominance of altruism", the physicist avers.

But why was one molecule, DNA, left over? "It is a mathematical result, a consequence of the dynamics of replicators", says Fontanari. He believes that there was the appearance of another kind of replicator: RNA, or ribonucleic acid, a simpler molecule (it is a single strand, while DNA is a double strand, like two intertwined threads). The idea gains strength with the proof that RNA manages to act like a replicator, creating copies of itself, and like an enzyme of another molecule. "DNA was an invention of RNA and of other more complex replicators", the physicist suggests. But it is the most recent invention that took the reins of evolution and, in the majority of organisms – save for a few viruses that store their genetic material in the form of RNA –, today it is DNA that makes RNA, in the initial process for producing proteins.

This set of ideas, which also helps one to understand why there is a single recipe for the production of proteins, the so-called genetic code, in any organism, contests the thesis of biological individualism, propagated after 1976 by the book *The Selfish Gene*, by English biologist Richard Dawkins. On the other hand, at no time does it run counter to Charles Darwin's principle of natural selection. "Nature does not need any organizing principle other than natural selection", he says.

The advantages of sex - Fontanari solved other impasses that biologists already knew about, but they were unable to explain with precision how they arose and developed. One of them is sexual reproduction. Scientists always wondered why sex can be an evolutionary advantage, particularly for organisms that enjoy both the alternatives – there are protozoa that can duplicate themselves with autonomy, without needing a partner, ensuring the continuity of all their genetic material, contained in the DNA, but which opt for sexual reproduction, by means of which they transmit only half of their genes. "There is a selective pressure in favor of the recombination of DNA", says Fontanari, who in this area is working with evolutionist biologists from the Middle Tennessee State University, United States.

Who raised the problem was American geneticist Hermann Joseph Müller (1890-1967), on discovering that X-rays cause mutations in fruit flies (*Drosophila melanogaster*), a finding that earned him the Nobel Prize for Medicine in 1946. Years later, there came the verdict: the mutations (changes in the DNA) do more harm than good, and they build up more rapidly in species that reproduce only in the asexual way, on a one-way road, which became known as Müller's ratchet. Müller himself suggested that sexual reproduction, for permitting the mixture of genetic material, could succeed in reversing the ratchet, and avoid the harmful effect of mutations, today seen as a source of diversity in living beings, but which – at least in part – threaten survival if not corrected, for continuously reducing the adaptation of animals and plants to the environment in which they live.

But an explanation was missing for the movement of Müller's ratchet, associated with phenomena that had been studied a lot, like the degeneration of the Y sexual chromosome. This was what Fontanari did in an article published in December 2001 in *Physical Review Letters*: the ratchet advances and the pawl moves, passing from one notch to another, when all the organisms of a population acquire the same mutation. It was already known that it is more probable for few mutations to occur than many – in a virus, at least one mutation per genome occurs at each replication.

Equations reveal the intensity of natural selection

The researcher from São Carlos closes the article with two formulas that, according to him, "have great potential for practical use", for determining the rate of mutation per genome and the intensity of natural selection, provided that the distribution of the adaptability of a species is known, measured by means of the frequency of individuals with different capacities for survival in one and the same environment. "If there were no mechanism like Müller's ratchet to show that microorganisms with asexual reproduction are at a disadvantage for not managing to annul mutation, it is the asexual forms that would predominate", he says. The same work shows why the ratchet does not stop, even though its movement may be slow. How slow? "It depends on the generation time of the organism involved", Fontanari replies. For bacteria, which create a new generation every 20 minutes, the ratchet will move a notch every 40 years, which corresponds to 1 million generations.

The elimination of mutations that ceaselessly alter the DNA, by means of the continuous production of new beings, can also be understood by means of an analogy with the Theory of the Red Queen, which refers to a character of the British writer Lewis Carroll in *Through the Looking Glass*. The Red Queen would not let anyone stop running, alleging: "We have to keep on running to stay in the same place". •

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