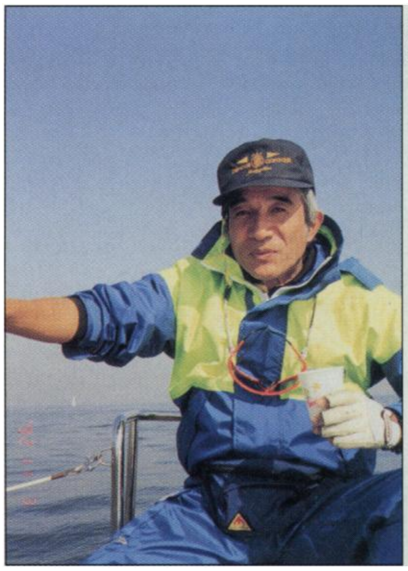
EVOLUTIONARY BIOLOGY

Impact of DNA Replication Errors Put to the Test

TOKYO—Molecular biologist Mitsuru Furusawa and embryologist Hirofumi Doi have spent the past decade pondering one of life's most basic processes: the replication of DNA. And they have come up with an intriguing idea. They have developed a theoretical model showing that the very process of DNA synthesis spreads mutations unevenly across a population. This process, they believe, may help keep populations robust by preserving a stable genetic baseline, yet pave the way for rapid evolutionary change when environmental conditions shift.

Furusawa, of Daiichi Pharmaceutical Co., and Doi, of the Institute for Social Information Science of Fujitsu Laboratories Ltd., have developed and tested their model with computer simulations. Soon, however, it may be put to a more realistic challenge: A team of Austrian scientists will look for the kinds of evolutionary changes the model predicts in two experimental organisms. If they succeed, the results will not only increase understanding of evolutionary processes but also elevate the status of evolutionary biology as an experimental science.

"I don't know what to expect from these experiments," says Richard Lenski, an evolutionary biologist at the Center for Microbial Ecology at Michigan State University, whose experiment in the evolution of *Escherichia coli* has stretched to 10,000 generations. "But what's exciting is getting informed molecular perspectives and informed evolutionary perspectives together to formulate and test some of these hypotheses." Biotechnologist Florian Rüker, of the Institute of Applied Microbiology of the University of Agriculture in Vienna, hopes that testing Furusawa's hypothesis will help him and colleague Gottfried Himmler solve an evolutionary puzzle: "It would explain how organisms can play around with their genes to make mutations while also ensuring they can survive a lethal mutation by keeping the wild type available," he says.



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In Darwin's wake. Furusawa ponders evolution aboard his yacht, *Beagle II*.

Furusawa and Doi's work, a major topic of discussion at a recent symposium here,* is based on the fact that the two strands of DNA's double helix are replicated separately. One strand—the leading strand—is continuously synthesized in the direction in which the helix unzips. The other—the lagging strand—is synthesized in numerous short fragments in the opposite direction. The two processes use different enzymes, and there are differences in the proofreading mechanism to correct errors. This dual procedure is known to introduce more errors in the lagging strand than in the leading strand, and Furusawa wondered whether the disparity might have broad evolutionary implications.

As head of molecular biology research at Daiichi, Furusawa didn't have the freedom to pursue that thought. But in 1987 he won a 5-year grant from the government-affiliated Research Development Corporation of Japan to investigate a number of his outside scientific interests. Furusawa and Doi used part of the grant to conduct computer population simulations to explore patterns of inheritance of these DNA replication errors. For simplicity, they treated these errors as "mutations." In one model—the parity model—they introduced errors in replication into both strands at the same rate. In the disparity model, more errors were introduced into the lagging strand than the leading strand.

Although the total number of errors introduced into the two model populations was the same, the distribution of those errors varied markedly after several generations. Errors in the parity-model population were spread uniformly among individuals, while in the disparity model there was much greater deviation from the mean: Individuals derived largely from leading-strand replication had many fewer errors, while individuals descended from the lagging strand had many more than the norm. The researchers believe it is particularly significant that while parity-model individuals with minimal numbers of mutations disappeared after several generations, disparity-model individuals with few or even no mutations survived down to the 15th generation.

Furusawa and Doi then linked the models to a computer optimization routine that assigned numerical "fitness" scores to different mutations. Individuals with fitness scores closer to an arbitrarily defined optimal score were deemed to have a better chance of producing offspring than were individuals with fitness scores further away from the optimum. Individuals with scores outside arbitrarily set limits could not reproduce. After several thousand generations, Furusawa and Doi found that disparity-model populations had "evolved" to higher fitness scores than had parity-model populations. They also evolved more rapidly. In addition, some parity models produced so many individuals with scores outside the limits for reproduction that the population died out, a fate that never befell the disparity models.

Furusawa and Doi believe these results point to a mechanism that ensures evolutionary changes will work to the benefit of a species. Because mutations do not spread evenly in the disparity-model populations, these populations are not only genetically more diverse, but individuals that accumulate very few mutations maintain a stable baseline for the species. As long as the baseline individuals have the highest fitness scores, they reproduce more and are not overwhelmed by less fit—and therefore less fecund—mutants. If mutants appear that have higher fitness scores, they become the new baseline. In contrast, the relatively even distribution of mutations throughout the parity-model populations eliminated the stable baseline.

Michigan's Lenski says he finds the computer simulations intriguing, but he questions how well they mimic the real world. Furusawa readily agrees, acknowledging that "there are no biological data to back this up." He is hoping, however, that such data may soon be available: As reported at the Tokyo symposium, two candidate organisms have emerged to test the theory.

One of those candidate organisms is a newly discovered mutant strain of *E. coli*, dnaQ49, which has a mutation rate 10,000 times greater than that of normal strains of *E. coli*, with replication errors occurring from 50 to 100 times more frequently in its lagging strand than in its leading strand. The second candidate is a yeast strain that demonstrates higher than normal mutation rates, although the discrepancy between the leading and lagging strands has not yet been checked.

Vienna's Himmler and Rüker intend to

* 4th Japan Research Development Corp. International Symposium, "Experimental Approaches to Evolutionary Biology," 6–7 March, in Tokyo.

subject these two strains to rapid changes in their environment by altering the temperature or the pH of their surroundings. Furusawa and Doi's theory predicts the disparity strains should evolve and adapt to the changes in the environment more quickly and successfully than do control strains.

One of the questions raised at the conference was why higher rates of mutation and greater discrepancy between the leading and lagging strand rates aren't the norm if they are more advantageous. Furusawa says one possibility is that nature may be conservative; unfortunately, that feature makes any lab test too slow to be practical. The planned experiments may not exactly imitate nature, but they are expected to yield useful data, especially if they involve additional mutants with differing ranges of mutation rates and differing degrees of discrepancy in mutation rates between the leading and lagging strand.

If Furusawa's hypothesis checks out, Himmler and Rüker see important implications for protein engineering. If disparity mutators do prove more adept at adapting, Rüker thinks it would set off a search to change other disparity mutators that might be teased into evolving to produce a variety of modified proteins and enzymes. Such an evolutionary approach promises to be more effective and efficient than current methods of producing mutant strains. To make this work, however, Rüker says they will also have to develop selection systems in which only strains that produce a wanted protein survive. "In the end, it is the practical application of evolutionary methods that is most important and interesting for us," Rüker says.

Furusawa hopes the Austrian experiment will generate so many mutations in a relatively short time that researchers will be able to see major changes develop fairly quickly. In fact, he sees the use of such strains that quickly generate mutations as the only way to "speed up evolution" and make possible further experiments.

–Dennis Normile

CLIMATE

Volcano–Ice Age Link Discounted

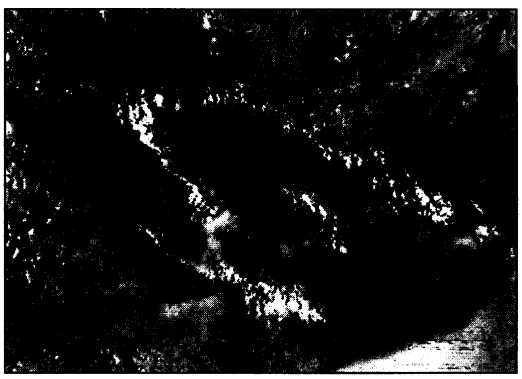
When the moderate-sized volcano Mount Pinatubo blew in 1991, it spewed enough debris into the stratosphere to dim the sun's warmth and cool the globe a few tenths of a degree for a year or two. So climatologists have long wondered how climate would respond to a truly massive eruption. As it happens, nature has already run the experiment: the great Toba eruption, tens of times bigger than any historical eruption, which exploded on Indonesia's island of Sumatra 70,000 years ago. And that's just when the Earth plunged into an ice age and the population of early humans apparently shrank catastrophically.

With such a tempting temporal connection, some scientists have speculated that the eruption caused an immediate "volcanic winter" that devastated early humans and also accelerated a long-term slide into the Ice Age. But researchers have long been frustrated in their search for a record of Toba's global effects. Now, in the 15 April issue of *Geophysical Research Letters*, Gregory Zielinski of the University of New Hampshire and colleagues report on such a record buried in the Greenland ice sheet—and they conclude that Toba wasn't such a major climatic catalyst after all.

By analyzing ice from the Greenland Ice Sheet Project 2 core, the researchers showed that Toba did indeed loft billions of tons of debris into the stratosphere. But correlation of that debris with climate records frozen in the same ice showed no climate effect during the millennia after the eruption. Toba "must have been a really spectacular eruption," says atmospheric physicist Brian Toon of Ames Research Center in Mountain View, California, but "climatewise, it's not obvious how much bigger it would have been than the two eruptions [of] the past 10 years."

But the Greenland ice record leaves little doubt that Toba packed a powerful punch. Geologists already knew that about 2800 cubic kilometers of magma must have been released, which dwarfs the largest eruption of historical times—the 50-cubic-kilometer eruption of Indonesia's Tambora in 1815. But it isn't the ash spewed in an eruption that cools the climate; ash particles are too large and fall out of the air too quickly. It's the sulfurous gases, which in the stratosphere convert to tiny droplets of sulfuric acid that form a persistent haze and block sunlight.

Analysis of Toba ash had suggested that the eruption loaded the atmosphere with 1 billion to 10 billion tons of sulfuric acid—100 times the release of Mount Pinatubo, although the numbers were controversial. But when Zielinski and his colleagues zeroed in on a sulfate spike in 71,000-year-old ice, they found that the earlier assessment was about right: Measuring the sulfate in a year-by-year record in the ice layers, they estimated that 2.2 billion to 4.4 billion tons of sulfuric acid fell around the world during 6 years.



LANDSAT

Big blast. Toba's erupton left a 100-kilometer caldera, but probably didn't start an ice age.

So there's no doubt that Toba put up plenty of sun-blocking haze. Michael Rampino of New York University and volcanologist Stephen Self of the University of Hawaii had suggested that the resulting cooling so enlarged the extent of snow fields and sea ice that even more sunlight was reflected back into space, pushing an already-cooling system into tens of thousands of years of ice age. But temperature records in the ice suggest Toba had no long-term effect.

The ice core showed a warming event before the ice age set in—and after Toba, says Zielinski. A strong warming between the eruption and the ice age "pretty much says Toba would not have had an impact on the 10,000-year scale of the ice age," he says. The eruption did occur within a 1000-year cold spell immediately preceding the temporary warming, but that cold snap had already begun when Toba blew, Zielinski says. At most, the eruption may have intensified the first few centuries of cooling.

Not everyone is willing to give up on Toba's catastrophic influence yet. "Greg is being correctly cautious," says Self. "I still think there's room for more evidence to come in. The next step is to start modeling to see what a Toba-sized atmospheric perturbation would do."

Rampino and Self had also suggested that Toba could have caused a sharper, briefer global cooling—of 3° to 5°C for a few years—and perhaps decimated early human populations in the process, as suggested by Stanley Ambrose of the University of Illinois. On this point the ice is silent for now: Zielinski didn't have enough ice left in his samples to extract an isotopic record of temperature on an annual scale.

But Toon notes that the huge mass of Toba's acid would not necessarily have translated into a proportionately huge climate effect. More acid means bigger acid droplets, which are less effective in blocking sunlight and fall out of the atmosphere faster. Toon estimates that Toba's acid haze may have been only a few times more opaque than Pinatubo's—not enough to trigger "volcanic winter." A firm answer on how much the human race suffered at the hands of Toba must await more clues from the ice.

–Richard A. Kerr