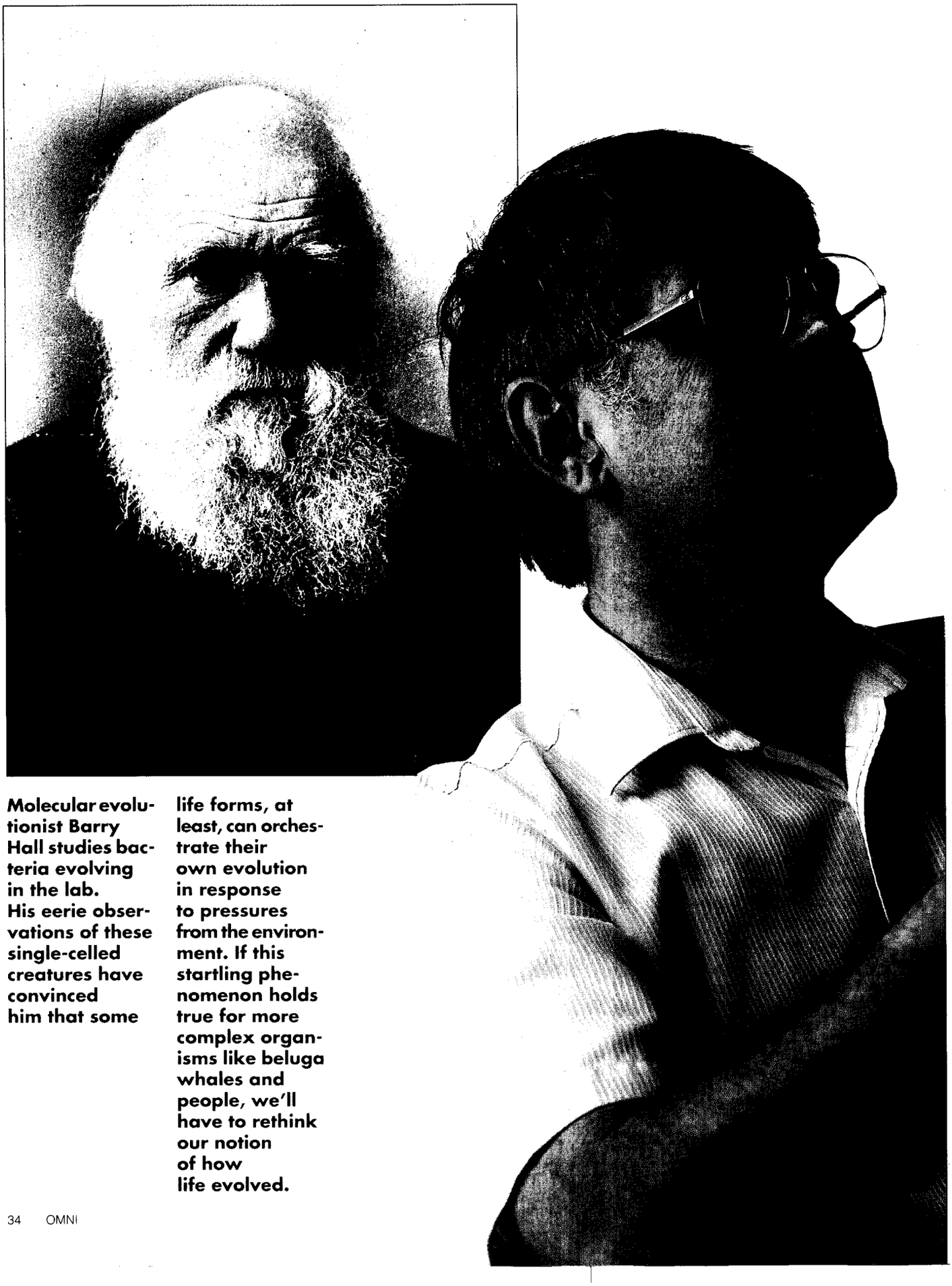
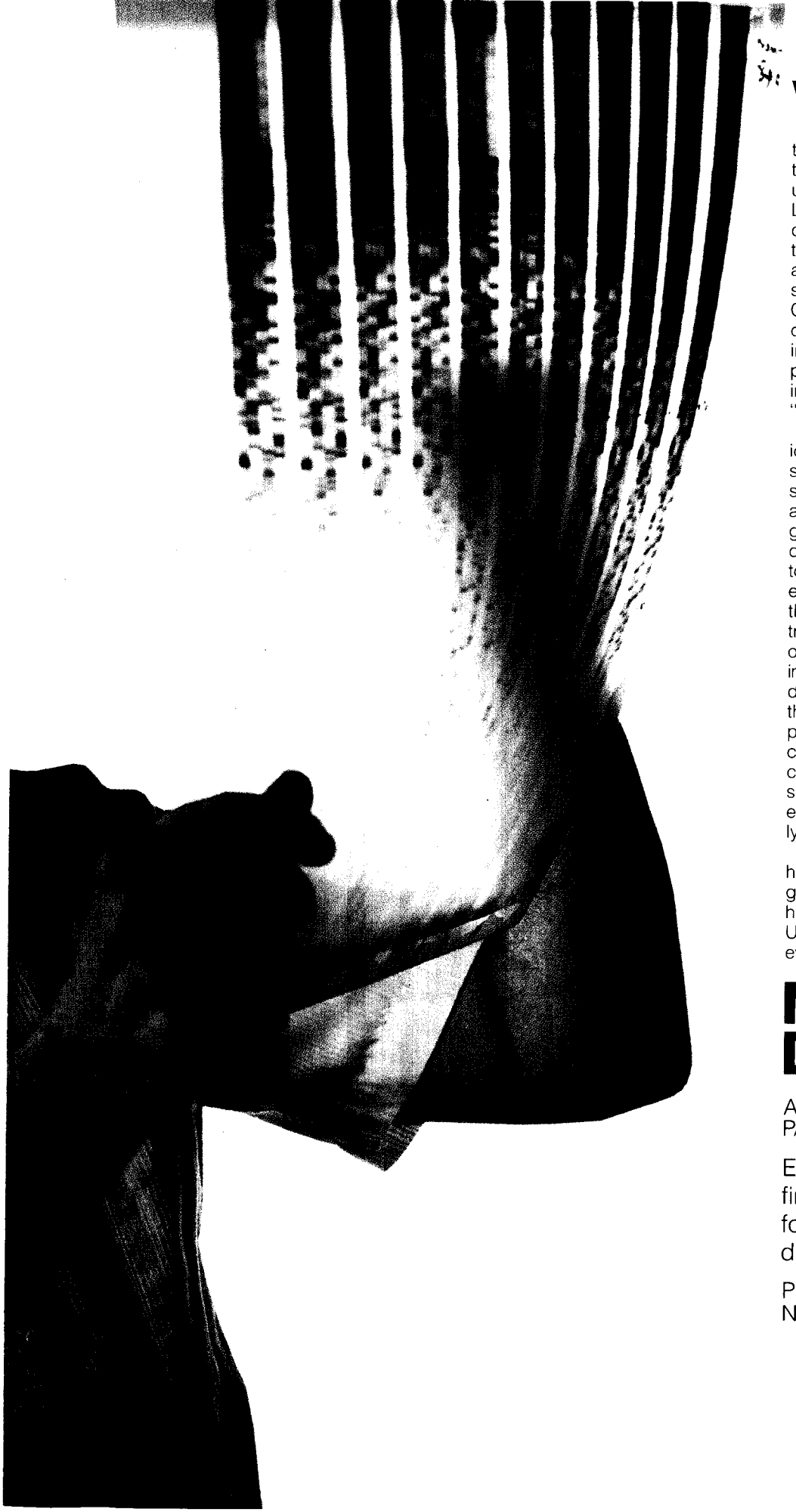


Molecular evolutionist Barry Hall studies bacteria evolving in the lab. His eerie observations of these single-celled creatures have convinced him that some life forms, at least, can orchestrate their own evolution in response to pressures from the environment. If this startling phenomenon holds true for more complex organisms like beluga whales and people, we'll have to rethink our notion of how life evolved.



When molecular biologist John Cairns arrived at Princeton for a debate in the summer of 1990, the mood was tense. The arena, a lecture hall at the university's esteemed Lewis Thomas Laboratories, boasted a wall-length chalkboard, overhead slide projectors, and comfortable seats with armrests for taking notes. But despite these academic trappings, Cairns could almost hear "the saloon doors swinging, the train approaching, and the wind rustling down the plains." Some of the spectators awaiting the debate had even dubbed it "the shoot-out on Main Street."

On one side of the dusty scientific road stood Bruce Levin, a professor from the University of Massachusetts at Amherst. Levin, like almost all biologists, believed that one elegant mechanism could explain the diversity of life on Earth. According to this prevailing view, all species evolve through random mutation of the genes. Populations with new traits arise when mutations produce organisms especially good at finding food, avoiding predators, and producing offspring. After generations, these successful mutants may replace earlier organisms within the species or even form whole new species. The process is called natural selection, since nature itself apparently selects the individuals most likely to survive.

Convinced of this scenario, Levin had come to Princeton, scientific six-guns at the ready, to stand down the heretics—Cairns and his colleague, University of Rochester molecular evolutionist Barry Hall. Not only had

NATURAL DIRECTION

ARTICLE BY
PAMELA WEINTRAUB

Extraordinary new findings suggest that life forms may literally direct their own evolution

PHOTOGRAPH BY
NEAL DAVIS

these two renegades challenged the prevailing orthodoxy, they had done so in *Nature* and *Genetics,* a couple of the most prestigious scientific journals in the world.

Cairns and Hall were not creationists who believed that people had been placed on Earth fully formed by heavenly design. Instead, they had come to Princeton with an alternative scenario for how evolution works: The mutations that drive evolution, the researchers claimed, were not always random. In experiment after experiment, they said, microorganisms seemed to be whipping up their own mutations—almost as if some inner molecular composer were helping the cells react to environmental requirements and needs. They even had a name for this shocking and powerful phenomenon: directed mutation.

At the Princeton conference, Levin argued against this radical view. "Mutants arise at random," he said, "whether they are favored by natural selection or not." Only *after* the mutants arise randomly, he added, does the environment kick in, with natural selection acting as the "editor of evolution," choosing the life forms it likes best among those already around.

Levin pointed out the technical limitations of the research, insisting that Cairns and collaborators had merely assumed directed mutation was occurring without sufficiently ruling out other, less radical explanations. To emphasize what he saw as the work's major flaw, he titled his talk "Refrigerator Lights and the Limits of Inductive Inference." The idea, he told the audience of professors and graduate students, was that no matter how many mundane explanations the researchers eventually disproved, there would always be more. Their approach, he stated, was a bit like trying to prove that a refrigerator light is off when the door is closed. "Even if you put a little kid in the refrigerator to tell you what's going on," he said, "you could never be sure the kid was telling the truth." They would never validate their theory, he concluded, unless they found the mechanism at its root.

But despite these objections, Cairns and Hall were impossible to ignore. As one seminar observer, Princeton graduate student Karen Weiler, recalls, "A lot of people there that night wanted to dismiss Cairns and Hall, but they just couldn't."

With good reason. The new research, if correct, would alter one of the most entrenched scientific theories of our time, in the process changing our notion of how life on Earth evolved. It might also explain the huge gaps in the fossil record—long epochs during which paleontologists can find no evidence of evolution at all. After all, if mutations are literally "directed" by life forms reacting to environmental change, then rapid evolution would occur primarily at highly "punctuated" moments—during ice ages, say, or meteor hits, when environmental stress is especially great. In fact, if the new scenario turns out to be valid not just for microorganisms, but also for more complex living things such as rain forests, animals, and humans, evolutionary biologists would have to rewrite much of the work they have labored over for the past hundred years.

Today's evolutionary biologists—often called "Neo-Darwinists"—base much of their work on the ideas of the master, Charles Darwin himself. While exploring the flora and fauna of South America and the Galápagos Islands in the 1830's, Darwin observed the immense variety of life. Even in a given species, there was large variation from one individual to the next. Based on this, Darwin proposed a brilliant theory for how evolution works: Nature was always generating variation, he declared, and in the brutal struggle to survive, some variants would just be more successful than others. Those better at exploiting the environment, he said, would have more offspring, and these individuals would prevail.

But despite this central insight, Darwin still didn't know why the variation occurred. The reason: The world had not yet heard of the tiny hereditary units called genes.

In the century after Darwin proposed his theory, however, biologists discovered that genes, found in every cell, determined the nature of living things. By orchestrating the synthesis of organic chemicals into the stuff of life, genes dictated virtually every biological characteristic from brain size to eye color and body type.

The genes themselves were composed of a helical molecule called deoxyribonucleic acid, or DNA. All DNA, in turn, consisted of just four chemical building blocks, called bases. These bases, strung together over and over like beads, could be arranged in literally millions of combinations, creating the potential for virtually infinite genetic diversity on Earth.

For a species to generate this diversity, said the Neo-Darwinists, all that was needed were some simple chance events. In the random shuffle of life, a few bases would accidentally be replaced by others. Over time, the accu-

"Mother was right. I should have left you years ago."

mulation of such changes—called "point" mutations because they occurred one base at a time—would result in different types of creatures, even whole new species.

The Neo-Darwinists who proposed this grand synthesis of Darwinian evolution and modern genetics said their theories were rooted in scientific fact. The first study to back them up was published in the 1940's, after Salvador E. Luria, then of Indiana University, began wondering how he could prove that mutations had occurred randomly and not in response to environmental stress. It occurred to Luria that he could compare genetic mutation to another rare event—hitting the jackpot at a slot machine in Las Vegas. People playing these slot machines usually came up empty-handed, Luria knew, but every so often, by chance, someone struck it rich.

Luria compared the slot machine to a colony of bacterial cells. Each cell reproduced by dividing in half. The two resulting daughter bacteria, in turn, reproduced by dividing in half again, and so on and so forth until, within a couple of days, one cell had become a swarming bacterial colony of 1 billion cells or more. If a cell were to mutate randomly, early in the life cycle of a colony, it would produce large numbers of mutant descendants, resulting in a "jackpot" of mutants.

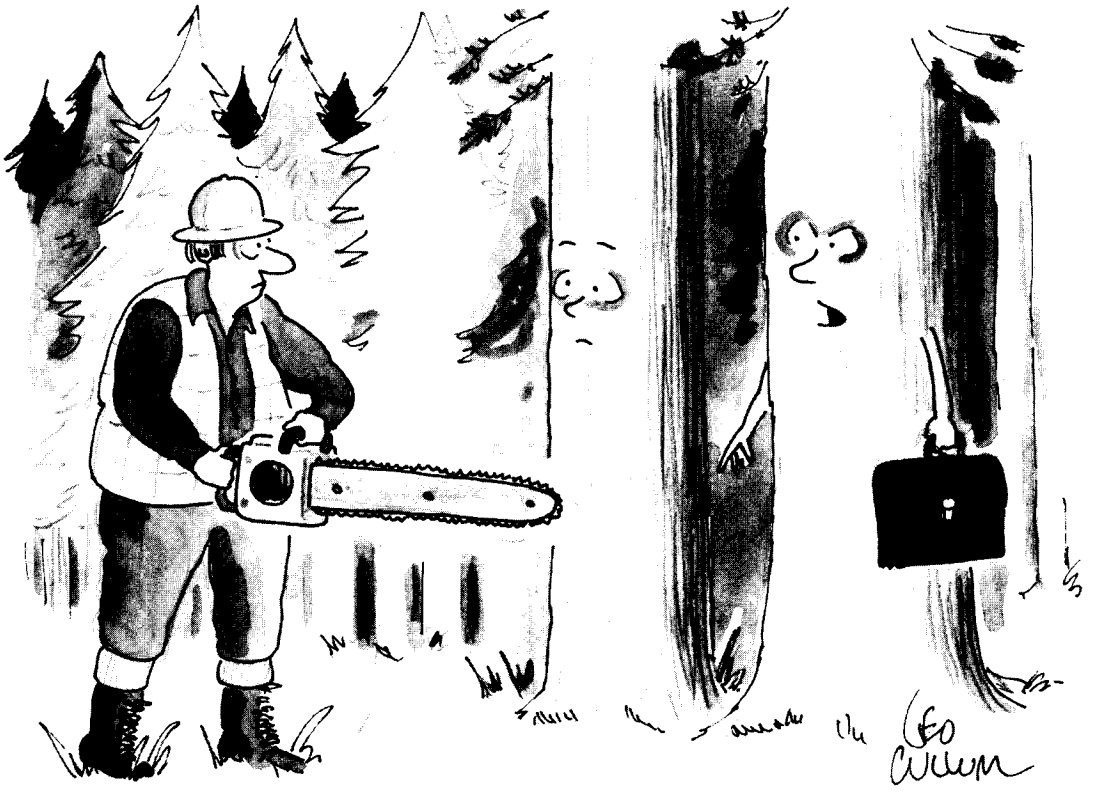
Using this concept as the basis for experiments, Luria and his colleague, Vanderbilt University physicist Max Delbrück, grew bacterial populations in test tubes. Then, after the populations had grown, the scientists introduced a lethal virus.

Mutants never seemed to emerge directly in response to the virus. Every so often, on the other hand, a given population just happened to contain huge numbers of mutants resistant to the virus. These mutants were so numerous they had obviously arisen early in the life of the population, way before the virus had been introduced, and represented jackpots of enormous proportion. The conclusion: Mutants resistant to the virus occurred randomly, without input from the environment. The environmental stress—in this case, exposure to a virus—came into play only later, selecting out the mutants that could survive.

If the Neo-Darwinists were happy to see their theories boosted in this way, they were even more overjoyed when, in the 1950's, geneticist Joshua Lederberg drove the point home. Lederberg started with a gel containing numerous colonies of bacteria. Then he pressed the gel onto a strip of velveteen as if he were printing on paper with a rubber stamp. Finally he took the velveteen and pressed it onto a second gel. When he pulled the velveteen off, the pattern of bacterial cells on the second gel mirrored that of the first. To do his experiment, Lederberg exposed only the bacteria on the second gel to the virus. A certain number of cells were immune to the virus, and only they survived.

The question was, did the resistant cells—mutants—develop in response to the virus, or were they there beforehand? To find out, Lederberg tested plate number one, and voilà: He found mutant cells resistant to the virus at the same exact site as on plate two. Obviously, the mutant cells had been there all along. Like Luria and Delbrück, Lederberg had validated the ideas of the Neo-Darwinists: Mutant organisms, he showed, emerged spontaneously, without any stimulation from the environment at all.

Satisfied with the evidence, the Neo-Darwinists spent the next 30 years refining their theories, coming up with all sorts of situations under which populations might evolve. But while they spent enormous time honing and polishing their theory, the overriding mechanisms—random mutation and natural



"Hold it! I'm his attorney!"

selection—remained the same.

And there the matter stood until the 1980's, when it fell under the scrutiny of British oncologist and molecular biologist John Cairns. A deeply thoughtful man with the regal good looks and bearing of actor Peter O'Toole, Cairns had spent years as director of the prestigious laboratory at Cold Spring Harbor in New York and was now in Boston at Harvard's School of Public Health. Interested in the mutations that induce cancer, he thought he might gain some helpful insight by studying mutations in bacterial cells.

Naturally, he began by going back to the old studies conducted by Luria, Delbrück, and Lederberg decades before. Examining their work more carefully, he realized that though they had conclusively proved the existence of random mutation, they hadn't ruled out other evolutionary mechanisms as well. Indeed, their crucial studies were plagued by an overwhelming flaw: the use of a *lethal* virus.

The virus presented a problem because, in bacterial populations, mutants resistant to virus take several generations to express themselves. The reason is that bacteria replicate by dividing and then growing. A first-generation mutant thus contains half of the cellular material from the nonmutant parent cell; in fact, new mutants carry so much parental material that they often seem to behave like the original strain. Only after many generations, when the original nonmutant gene products have been diluted out, can the mutant's new characteristics truly emerge.

Therefore, in the Luria and Delbrück experiment, the very virus that might have *caused* the production of resistant mutants would have killed those mutants instantly. Mutants produced in direct response to a lethal virus would never be observed.

To get around this problem, Cairns decided to see if he could generate mutants through a less instantly lethal form of selection: He would deny his bacteria access to all nutrients except for one that they lacked the ability to use. Either they would learn to use the new nutrient, or else they would eventually starve to death. "The question was," Cairns explains, "could some mutants arise as a result of pressure from the environment?"

He began his experiment with populations of bacteria unable to digest the sugar lactose. Then he placed these bacteria in a medium that contained only lactose for a food source. Of course, the bacteria stopped multiplying because they had no usable food. But after a few days, large numbers of the lactose-utilizing mutants began to appear. The mutants were so numerous, in fact, that they could not be accounted for by the theory of strictly random mutation. The suggestion: The bacteria were learning to generate their own, useful mutations through a surprising evolutionary process that wasn't random at all.

Cairns published his study in *Nature* in 1988. Near the end of the article, he suggested some ways in which the environment could influence genetic material, thus allowing directed mutation to occur. Each of these suggested processes, Cairns had the chutzpah to write, "could, in effect, provide a mechanism for the inheritance of acquired characteristics."

The statement inspired sentiments of fear and loathing among evolutionary biologists worldwide. The term *acquired characteristics,* after all, smacked of the discredited eighteenth-century biologist Jean-Baptiste Lamarck, who proposed that evolution proceeded as individuals used various organs, muscles, and limbs. For instance, Lamarck had declared, if a creature under stress was forced to exert extreme muscular strength, offspring would inherit—or

"He's trying to determine why the same biological process can produce both a Democrat or Republican."

acquire—larger muscles whether or not they actually required the additional strength. Cairns had used the phrase "acquired characteristics" by way of analogy *only;* he was talking about genes and proteins, not fingers and toes. But that didn't stop his critics from writing to *Nature* in droves. They insisted that Cairns tighten his laboratory controls and proposed alternative scenarios that would leave the Neo-Darwinian interpretation intact.

But Cairns stood philosophically firm: "It's easy to imagine molecular mechanisms that *might* drive the process of directed mutation," he explained. "We've already proven feedback between organisms and the environment; this occurs through messenger molecules that help genes communicate with the cell and the outside world." In light of this, he added, "It seems almost perverse to maintain, as a matter of principle, that evolution is driven only by random mutations, and that no other phenomenon comes into play."

One researcher wholeheartedly agreed. Molecular evolutionist Barry Hall had been on a similar track for years. His involvement in the field began in 1970, while visiting his good friend, University of Minnesota population geneticist Dan Hartl. Hartl had been studying the fruit fly *Drosophila,* monitoring how large groups of these creatures evolved from one generation to the next. Hall, on the other hand, was studying the general molecular and cellular biology of the popular laboratory bacteria *E. coli.* "We got to talking," Hall explains, "and kind of said, Gee, wouldn't it be nice if you could watch evolution as it happened, on the molecular and cellular level, by experimenting with bacteria?"

A couple of years later, Hall began the work. He started with a strain of bacteria normal in all respects but one: The individuals in his colonies lacked an enzyme necessary for digesting lactose. He plated bacteria from this strain on a dish containing a blood-red gel known as a Macconkey medium. Dissolved in the medium were two types of food sources: a small amount of peptide and a large amount of lactose. The gel was an important indicator, since bacteria that digested lactose would absorb some of the dye, showing up as red; those that digested the peptide would not absorb the dye and would thus appear white.

The bacteria, unable to digest the lactose, consumed all the peptides. As they grew, they peppered the blood-red expanse of Macconkey with white colonies. When all the peptides were gone, bacterial growth seemed to stop. But out of curiosity, Hall let these seemingly stymied colonies sit around his lab for a week or two. In every case, he found, pimples of red began bursting through the islands of white. These red bursts, called *papillae,* were new colonies of bacteria, now able to utilize the lactose. In short, they were mutants.

For nearly a decade, through stints at the University of Newfoundland and the University of Connecticut, Hall watched his bacteria give rise to mutant offspring capable of digesting lactose. As he performed the experiments, he began to realize the oddness of his results. Time and again, his bacteria were evolving the ability to eat the lactose about a hundred million times more frequently than would be expected if mutation had occurred purely by chance. What made the results especially strange was the magnitude of the genetic change involved. Sequencing the bacterial genes, Hall discovered that two genetic mutations, not just one, were required for digestion of lactose to occur.

Hall discovered the phenomenon in other *E. coli* populations as well. He was absolutely floored, for instance, when

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"I'd say the left side of your brain is shot to hell."

DIRECTION

CONTINUED FROM PAGE 41

he used his technique to create *E. coli* mutants that could thrive on the carbon source citrate. "This was weird," he says, "because one of the definitions of *E. coli*, one of the things that's used to distinguish it from all other closely related organisms, is that it cannot use citrate." Mapping the genes of his citrate mutants, Hall found "the improbable stacked on top of the highly unlikely" when it turned out that the citrate-consuming *E. coli* had two large-scale genetic mutations, not just a single altered base. The finding was so completely out of line with results predicted by accepted evolutionary theory that Hall didn't know what to think. "At that point," he recalls, "all I could do was throw up my hands." Yet by 1988, when Cairns described the phenomenon of directed mutation in *Nature*, Hall realized that he had been studying this phenomenon as well. By then at the University of Rochester, he had witnessed directed mutation in thousands of bacterial colonies and had charted its course in many specific *E. coli* genes. He was also beginning to study the phenomenon in yeast.

Discussing the research today from his immaculate Rochester office, his spanking new lab overflowing with projects next door, Hall expresses awe at the mysteries he has seen. "For almost fifteen years," he says, "I have been slapped in the face with the highly improbable. When that happens, you either get religion and say, 'God is favoring me,' or you conclude that perhaps your understanding of the process—in this case, the process of evolution—is incomplete."

Hall did the latter. Paying attention to his organisms, the lowly bacteria, he has been able to reach just one conclusion: "While some mutations may be random, many others are generated by the organism to cope with environmental stress." Because these mutations are literally selected by the organism while it is under stress, Hall calls them "selection induced."

To date, Hall has generated selection-induced mutations for half a dozen *E. coli* genes and a couple of yeast genes as well. Most of the time, he worked with bacteria unable to utilize nutrients such as lactose. He has also worked with bacteria unable to replicate because they lack the ability to manufacture critical amino acids, the building blocks of protein. When he first places these bacterial strains on a plate or in a liquid medium, the cells seem to stop growing. But after a few weeks, Hall finds large numbers of mutants that *can* utilize the nutrients or manufacture the needed amino acids.

In dozens of control studies, moreover, Hall has shown that the mutants are specific to the environment. The starving cells do not just start churning out mutants at random. If lactose is the only nutrient available, for instance, the mutants will develop the ability to digest only lactose, not some other, unrelated sugar. If the medium is missing the amino acid tryptophane, then the cells will evolve the ability to produce that amino acid only.

These days Hall and Cairns regularly correspond. One of their most pressing concerns: Figuring out how bacteria and yeast can possibly "know" what mutations to make. As Hall himself says, "It's implausible that a single cell has an array of machinery complex enough to measure the environment and then, in effect, say, 'Oh, *this* is how I have to mutate,' and then just go out and do it. Yet that is what seems to occur."

No viable theory has yet emerged, though Cairns and other researchers have speculated on the existence of something like a spontaneous mutation generator. "Imagine," says Cairns, "that these guys [the bacteria] are out there struggling, and they're not multiplying because of the stress. Mutations are spun out and then gotten rid of, until finally one is good. The lights go on, the dynamo starts humming, and the cell can grow. At that point the muta-

tion generator comes to a halt."

No matter what the mechanism, however, one question dominates: Even if this eerie phenomenon plays a large role in the evolution of microorganisms, does it have a similar impact on the human species? Evolution, Cairns believes, works the same way for the simplest, one-celled organisms and the most complex. No matter what the life form, he says, "the process is the same." Adds Hall, "As organisms evolve, they affect the environment. The environment, in turn, has an impact on life. If directed mutation turns out to be a powerful evolutionary force, we may have to reanalyze the feedback loops between the biosphere and the earth."

But Levin of Amherst insists that, despite the elegance of some of the work, it is not strong enough to stake a claim. "Until Cairns, Hall, and others show the mechanism by which directed mutation takes place, I will be skeptical," he states. "They certainly haven't shown that organisms perceive the environment and then understand what they need, nor have they demonstrated that organisms have the cellular machinery for this perception."

Some of the strongest criticism to date has been offered by evolutionary biologist Richard Lenski of the University of California at Irvine. Working with graduate student John Mittler, Lenski has recently published a paper in *Nature* himself. According to Lenski, cells may simply generate large numbers of certain types of mutations when they are starved, as Cairns and Hall's cells are. He also suggests that some bacterial populations may increase in number by literally consuming bacterial waste products; with more cells in the population, one might *expect* to find a larger number of mutants.

Hall, for his part, counters that he continues to test all possible explanations for directed mutation in his lab; as critics suggest additional control possibilities, he says, he will test those as well, "no matter how foolish they seem." None of the explanations posed so far, he adds, come close to explaining the effect, at least according to his painstaking control studies in the lab. To bolster his argument, he takes out a stack of papers currently in press and reams of data from his shelves. Drawing furiously on his chalkboard, he seems to demolish the notion that cell starvation or an undetected increase in colony size can account for the numbers and types of mutations he has seen.

Cairns, soon to retire to his native England, says that the critics "see themselves as crusaders defending some religion, and by hook or by crook, defend it they will. But the world will pass them by." The reason, Cairns notes, is the power of science itself. "Our studies are ever more detailed," he says, "and system after system seems to be demonstrating this effect. The data will speak for itself."

If that data holds, evolutionary biologists will have to go back to the drawing board and rewrite their theories of how earthly life evolved. If directed evolution turns out to affect not just microorganisms, but also more complex living things, then we may have to reanalyze the fossil record and revamp the history of *Homo sapiens* as well. Says Hall, "It would require a paradigm shift in the way we view the world."

Whichever way the evidence finally points, however, it will be business as usual for lab hound Barry Hall. "The Neo-Darwinists claim that evolution works too slowly, and on such large populations, it's simply impossible to study the process," he concludes. "But for people working with bacteria it is possible to study evolution as it happens. Biology is an experimental science, not a theoretical one. The business we're involved in is asking—not telling—the universe how it works."