PROFILE

Profile of Bruce Levin

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For population biologist Bruce Levin, opening the incubator to see how yesterday's experiment worked is his ideal way to start the day. That anticipation has kept the Emory University professor of biology excited about his research on the population and evolutionary biology of bacteria for more than 40 years, and motivated a shift in interest from multicellular organisms to bacteria. Anything that takes longer than a day to see results, says Levin, takes too long.

Levin uses mathematical and computer modeling and experiments with bacteria to investigate basic ecological and evolutionary questions. He employs the same techniques to study health-related problems, including the epidemiology and evolution of pathogenic bacteria and the population and evolutionary dynamics of antibiotic treatment and resistance.

Elected to the National Academy of Sciences in 2012, Levin uses mathematical and computer simulation models to determine the optimal way to use antibiotics to treat acute infection, as described in his Inaugural Article (1).

"Hopped Up" on Research

Levin grew up in the Bronx, New York, and his parents hoped he would pursue a career in medicine. Not cut out for medicine, Levin decided to major in engineering when he was accepted at the University of Michigan, Ann Arbor.

"Engineering was legitimate for my parents, though not as legitimate as real doctoring," says Levin. However, engineering was not what he had expected. "My vision was that of a 19th century inventor. It was a very romantic idea and not at all like what I was learning in school."

After his second year, Levin switched to zoology. The subject matter was interesting, but he was no more motivated as a student. He soon discovered the joy of research during an introductory genetics class taught by Morris Foster. Levin wanted to avoid writing the required term paper and decided to leverage the computer programming skills he had gained as an engineering student. He asked Foster whether, instead of writing the paper, he might work on computer simulations of genetic phenomena.

Bruce Levin. Image courtesy of Emory University Photo/Video.

"Dr. Foster was what a teacher should be," recalls Levin. "He was totally supportive of me, a hardly stellar undergrad. He introduced me to two graduate students working on mice, Mike Petras and David Rasmussen, and gave me a desk in a room I shared with some 1,000 deer mice. With Mike and David, I did my first research, a computer simulation of the population genetics of a lethal gene complex in house mice."

"Once I saw how much fun research was, I was totally hopped up and saw no choice but to go to graduate school," he says.

Levin stayed at Ann Arbor for graduate school. Thanks to his programming skills, he met William J. (Jack) Schull who agreed to be his PhD advisor, despite their differing interests. Levin wanted to become an ecological geneticist focusing on *Drosophila,* and Schull was a mathematical-statistical geneticist working on human populations. "Although I had my own lab and worked independently of him, Jack was always there when I needed advice. He still is, even now at 92," says Levin. "He was extremely supportive of me and taught me to be the same with my students and postdocs."

Switching to Bacteria

As a graduate student, Levin decided he would integrate population genetics and population ecology. At the time, in the mid-1960s, ecology was concerned with the distribution and abundance of species and population genetics was focused on the genetic basis of evolution, and the two spheres rarely overlapped. He achieved his goal, but only after he switched focus from *Drosophila* to bacteria, moving from Ann Arbor to Brown University as an assistant professor after completing his PhD in 1967.

Working with *Drosophila* was unsatisfying, says Levin. He could not control the environment, and evolutionary experiments were long and difficult to repeat. With bacteria, in particular *Escherichia coli*, he could perform ecological and evolutionary experiments in real time and test hypotheses within days under well-controlled and reproducible conditions.

Brown colleagues Seymour Lederberg and Frank Rothman made his transition from

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 8331.

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Drosophila to *E. coli* seamless, and within about eight weeks he had enough data to publish a paper in *Science* (2). In the paper, he presented evidence that two strains of *E. coli* could stably coexist on a single limiting resource, glucose, contrary to the prevailing view that the number of coexisting species had to be less than the number of resources.

"It was an important result at the time," he says. "But as I went on with my research I realized that the resource-species hypothesis was untestable. Even though glucose was the limiting resource, once you put bacteria in there are an abundance of metabolic byproduct resources to share."

While at Brown, Levin met the person he describes as "the most awesome mathematician and human being with a real interest in biology": Frank M. Stewart, with whom he collaborated from the early 1970s through the 1990s. Their first paper together (3), published after Levin left Brown for the University of Massachusetts at Amherst in 1971, was motivated by the results of the *Science* paper but was purely theoretical. Then, in 1977, along with Stewart and his first graduate student, Lin Chao, Levin conducted a study that would serve as the model for much of Levin's career: combining mathematical and computer simulation modeling with in vitro experiments with bacteria to estimate the parameters of the models and test hypotheses generated from the analysis of their properties (4).

Bacteria for Bacteria's Sake

When Levin initially switched to bacteria, he was concerned about extrapolating his findings to higher organisms. Soon, however, he realized there was a lot to learn from studying bacteria for bacteria's sake.

In the early 1980s, along with Robert Selander, an evolutionary biologist now at Penn State University, Levin studied the population genetics of *E. coli* from natural sources. These studies were among the first of what is now known as molecular epidemiology, including an 11-month study of the genetic diversity of the *E. coli* from Levin's own gut (5) and a study of the distribution of *E. coli* genotypes within and between families, including Levin's pet cat and dog (6).

In 1983, Levin's now-close friend Michigan State University molecular biologist Richard Lenski joined his laboratory a postdoc. "It was a joy to work with Richard," says Levin, "three-plus years of the most delicious scientific arguments." One of their joint articles, an experimental study of coevolution in bacteria and their viruses, won the 1985 prize for the best article in the *American Naturalist* (7). "Ecological and evolutionary studies with bacteria were becoming legitimate," says Levin.

Levin also began studying the population dynamics and evolution of infectious disease. Many of these studies examined how infectious diseases evolve both within a host and over time among many hosts, asking questions about how virulence evolves and is maintained.

A paper with his colleague and friend Jim Bull, an evolutionary biologist at the University of Texas at Austin, postulates that for some pathogens, such as poliovirus, virulence is the product of short-sighted evolution within hosts (8). The morbidity and mortality of infections are also often due to host failings, says Levin. A paper he wrote with his MD/PhD student Elisa Margolis (9) argues that virulence commonly results from the host's runaway immune response.

"Things like sepsis are examples of the downside of our immune system," says Levin.

"I believe that the future of treating infections is not going to be with antimicrobial drugs but rather by controlling the immune over-response."

Linking basic research and theory in ecology and evolution to applications in medicine have become a common theme in Levin's work. Since leaving Massachusetts for Emory University in 1992, much of his work has focused on antibiotic treatment and resistance.

"The motivation behind these studies is a desire to make a real contribution to human health and well-being," says Levin. "I have been particularly fortunate in this enterprise to work with a phenomenal postdoc Marc Lipsitch, who is now a professor at the Harvard School of Public Health, and my now 'brother' Fernando Baquero, a brilliant microbiologist and physician from Madrid."

Levin's Inaugural Article (1) with MD/PhD student Peter (Pierre) Ankomah uses mathematical and computer simulation models to explore antibiotic use protocols that can simultaneously minimize the term of acute bacterial infections and the likelihood of resistance emerging during the course of treatment. The model, which includes the contribution of the immune response, predicts that full-course high-dose treatment is the optimal way to achieve these ends. "We are careful to point out that this is a theoretical study, but one that generates hypotheses that can be tested," says Levin.

The "CRISPR-Cas Biz"

Although antibiotic treatment and resistance studies continue to dominate Levin's research, recently he has been studying an adaptive immune system that abounds in bacteria and archaea, called CRISPR-Cas. CRISPR are short sequences of repeated DNA separated by DNA commonly acquired from bacteriophage and plasmids. The CRISPR-Cas system helps bacteria abort infections by phage or plasmids that contain DNA identical to the acquired sequences, providing protection against lethal phage. However, the system can also prevent bacteria from acquiring potentially beneficial genes borne on plasmids and temperate phage.

Levin's first contribution to the "CRISPR-Cas biz," as he calls it, was a theoretical study of the conditions under which this bacterial immune system would evolve and be maintained (10). Along with North Carolina State University molecular biologist Rodolphe Barrangou and Université Laval microbiologist Sylvain Moineau, he examined the yogurt bacteria *Streptococcus thermophilus* and its phage (11). "This study is a superb example of why one has to do the experiments and how one learns most from models when they don't fit," says Levin. The molecular biology findings were accurate, but their model of the population dynamics of phage was not. The bacteria had unanticipated tricks that enabled them to survive phage infection without CRISPR and produced compounds that prevented CRISPR-protected cells from becoming established.

Along with Rockefeller University molecular geneticist Luciano Marriffini, Levin has been examining the inadvertent drawback of the CRISPR-Cas system: preventing the acquisition of beneficial DNA. Their first study found that when they offered *Staphylococcus epidermidis* an antibiotic resistance plasmid needed for their survival—which CRISPR-Cas prevents them from receiving—the bacteria lose CRISPR-Cas (12). The result provides a possible explanation for the extraordinary diversity in the existence and function of CRISPR-Cas and for the fact that drug-resistant strains of pathogenic bacteria commonly lack CRISPR.

Levin describes his studies with CRISPR-Cas as another example "of the joy of this enterprise and the utter privilege of being able to work on delicious questions with great people."

Although he continues to work at the bench as well as on a keyboard programming, Levin increasingly sees himself as a producer, kibitzer, and advisor for the research in his laboratory. Nevertheless, he feels the same

sense of excitement as he opens the incubator to see the results of yesterday's experiment as he did at the start of his career. "After all these years, I still can't wait to start my 'work' day. I can't imagine a better and more fulfilling lifestyle," he says.

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