evolution revolution

Story by Sona Pai
Photo by Lincoln Barbour

By observing generations upon generations of a tiny worm, MU alumnus Dee Denver could pave the way toward new treatments for cancer.

At about a millimeter long, the shape of a string bean and barely the size of a comma, the roundworm *Caenorhabditis elegans* has thousands of admirers across the globe. It's the subject of books and Web sites, conferences and consortia. It's been tapped for several space missions. It even helped win Nobel Prizes in 2002 and 2006. In the soils of most temperate regions on earth, *C. elegans* is just one of at least 80,000 species of nematodes and one of billions of creatures that squirm beneath our feet every day. In the scientific world, however, *C. elegans* is a rock star.

"It's beautiful," says Dee Denver, BS '96, a research scientist at Oregon State University. "It's the worm everyone wants to work with."

In the wild, *C. elegans* prefers to hang out near compost heaps or other decaying vegetable matter where it's most likely to find its favorite food: bacteria. Its life is short — only about two to three weeks — and one generation gives birth to the next every four days. Most *C. elegans* worms are hermaphrodites, meaning one worm can generate its own offspring without introducing another worm's genetic material.

In the lab, *C. elegans* can be found in Petri dishes, under microscopes and in freezers. Like the fruit fly and the common laboratory mouse, scientists consider *C. elegans* a model organism they can study to learn more about human genetics and physiology. With its transparent skin, which allows for easy viewing of internal processes, its short generation time, its simple organ systems and its single-parent reproduction, *C. elegans* is more like a supermodel organism. Among multicellular organisms, its genome was the first to be sequenced, and scientists have used it to study everything from nicotine withdrawal to aging to nutrient absorption in space.

> Imagine how physicists would react to learning that the speed of light is actually 10 times faster than they thought.

For the past 10 years, Denver has bred thousands of the tiny nematodes to study their rate of genetic mutation from one generation to the next. So far, his research has shown that genetic mutation occurs at a rate 10 times faster than scientists previously thought, causing a stir in the scientific community, requiring updates to classroom textbooks, and earning Denver the distinction of being one of the top young geneticists in the country. His basic research could lead to a better understanding of how cancer cells mutate in the human body and how to stop them from spreading.

"If we could reduce the mutation rate, we might reduce the incidence of cancer," Denver says. "Rather than trying to create a specific drug to treat a specific problem, we could find a way to prevent the problem from happening in the first place."

For example, scientists could reduce the mutation rate by designing genes that encode enzymes to repair damaged DNA, or enzymes that better replicate the DNA molecule to prevent mutation from happening. Denver says these possibilities are "the stuff of fantasy" for now, but they are theoretically possible. His work with a tiny worm is a critical first step in the process.

Geneticist Dee Denver collects nematodes from rotting apples at an orchard in Corvallis, Ore. The compost-loving worms could provide clues about the role of genetic mutation in human disease.


BD

Image courtesy of Pasquinelli Labs

Transparent skin, simple organ systems and asexual reproduction make *C. elegans* an ideal model organism for scientific research.

Small subjects, big questions

Denver's interest in nature and its workings started small. As a child growing up in St. Joseph, Mo., he loved to play outside, explore and dig in the dirt. "I may not have been thinking about science at the time, but I was interested in discovery," he says. "I liked turning over rocks to see what crawled out. It was exciting to see that there was so much happening at a much smaller level."

In the fall of 1992, Denver began his freshman year as a chemistry major at MU, where he met his future wife, Stephanie Swenson, BS Ed '96. The following year, he changed his major to biology with an emphasis in genetics. Still not sure of his career aspirations, Denver pursued what he found most interesting: exploring the unseen and investigating the unknown.

To help pay for school, he worked as a lab assistant on campus, helping with faculty member Wade Welshons' research on how the breast cancer drug tamoxifen affects growth rates in tissue cells. Although Denver was an hourly employee, washing beakers and helping out with other daily tasks, he got a feel for the lab environment, and he liked what he saw.

"I got to see, on a daily basis, how science worked in the lab setting, at the basic level," Denver says. "I liked the independence scientists had to come up with questions that interested them and then find ways to answer them. I was also kind of fascinated by the way basic research could help answer big questions — how studying something as fundamental as cells could play a role in larger, complex issues of life and death."

After graduating from MU in 1996, Denver moved to Kansas City, Mo., where he worked in research and development at a spice company, mixing new spice combinations and analyzing them for the nutrient content information found on product labels. Although the work was steady and paid well, he missed the thrill of investigation and exploration he had seen as an undergraduate. In his free time, he continued learning more about genetics and evolution. He read studies in research publications such as *Science* and *Nature*, and he began seriously considering a career in basic research.

"It takes a lot of confidence and a lot of persistence to do that kind of work," Denver says. "Sure it's exciting to think about answering unanswered questions, but they're unanswered for a reason — they're tough. People might work for five years before getting any kind of result."

Denver entered a doctoral program in the School of Biological Sciences at the University of Missouri–Kansas City, where he began working with the subject that would be central to his work for the next decade: *C. elegans*.

The worm leads the way

In the cells of every organism, DNA exists in two places: the nucleus and the mitochondria, the cell's power source. DNA itself consists of a series of nucleotides — adenine, thymine, guanine and cytosine — that line up in pairs to form the two strands of the DNA double-helix. The order and frequency of these base pairs determine the genetic blueprint that informs every cell in the new organism. As cells divide during reproduction, the DNA within them must replicate itself in a process similar to retyping a manuscript letter by letter. Replication "typos" cause changes known as genetic mutations, which make the new organism slightly different from its parent.

During sexual reproduction, DNA from two different organisms combines to form a third organism that is similar to both parents but exactly like neither. Because of its hermaphroditic reproduction, *C. elegans* lets scientists study genetic mutation in its pure state — the "natural" errors that occur when one cell tries to copy itself identically, without incorporating additional genetic information from another parent.

Although some mutations can improve an organism's chances of survival, most mutations are detrimental, causing disease, malformations or other negative effects. In a natural environment, natural selection makes mutations difficult to measure.

"If a mutation happens that causes fewer of a worm's offspring to survive, that mutation would be eliminated within a few generations because the worms with it would die off," Denver says. "If you can't detect mutations, you can't get an accurate understanding of the spectrum and rate of mutation."

To get a clear picture of how often mutations occur, Denver knew he had to remove the variable of natural selection. As a doctoral student at UMKC, he worked with scientists at Indiana University who had bred hundreds of generations of *C. elegans* over three years in a process that gave every worm an equal shot at surviving. When one worm reproduced, they chose a random sample of 100 of its approximately 300 offspring and placed each one in its own Petri dish. When those worms reproduced, researchers selected one offspring at random from each and placed it in a new Petri dish, and so on. This created a set of 100 independent genetic lines of *C. elegans* in which worms with genetic mutations had the same chance of surviving as those without.

"I had always thought that textbook calculations were underestimated because they didn't account for mutations that had been weeded out by natural selection," Denver says. "Previous studies had been done more quickly, but the calculations were incredibly complex, and the results were full of asterisks and caveats because there were so many variables they couldn't rule out."

Using expensive DNA sequencing technology, Denver looked at mutations in the worm's mitochondrial DNA — a more

affordable 13,000 base pairs compared with the 100 million found in the nucleus. By comparing the DNA of the worms in the 250th generation with the DNA of the first-generation worm, he found a rate of mutation that was 100 times faster than previous estimates. In 2000, the journal *Science* published his findings.

"I think people thought the results were interesting, but that they were probably unique to mitochondrial DNA," he says. "The real action is in the nucleus."

After earning his doctorate at UMKC, Denver moved on to Indiana University to continue his work as a postdoctoral fellow with the *C. elegans* researchers he had been collaborating with. By then, the original *C. elegans* lines were in their 450th generation, and DNA sequencing had become more affordable. Denver applied the same techniques he had used with mitochondrial DNA to the worms' nuclear DNA. This time, he found a rate 10 times faster than textbook estimates.

Denver's work challenged what had been considered a given in genetics research, and if verified by additional research, it would have a ripple effect through countless other studies — imagine how physicists would react to learning that the speed of light is actually 10 times faster than they thought. In 2004, when Denver's findings appeared in *Nature*, the scientific community and the media took notice. National Public Radio and other media outlets came calling, and in October 2007, *The Scientist* magazine identified him as a "scientist to watch."

"It definitely ruffled some feathers," Denver says. "The results went against a hundred years of previous work, which a lot of subsequent work had been based on. Some people think it might be unique to this species, and some simply don't believe the results, but no one has pointed out a flaw in the [study's] design."

Although random, spontaneous mutation has long been known as a potential source of cancer, the previous low estimates of the mutation rate made its role seem negligible. Denver's rates are high enough to suggest that spontaneous mutation could play a more prominent role than scientists previously thought.

Looking at the cause of cancer and other diseases through an evolutionary lens could reveal new possibilities for treatment. Denver says this is already happening with the flu vaccine: Scientists study how flu strains evolve over the course of a year to help them design the vaccine for the following year.

"It just makes sense to look at disease from an evolutionary perspective," he says.

Looking at the cause of cancer and other diseases through an evolutionary lens could reveal new possibilities for treatment.

"We learned that with antibiotics — the chemicals used to kill bacteria. From the 1970s to the 1990s, antibiotics were prescribed willy-nilly for almost anything."

Denver says that initially, the medical community thought of antibiotics as "wonder drugs" that could be used to kill all harmful bacteria. But they failed to realize that some bacteria carry a beneficial mutation that makes them resistant to antibiotics. Those are the bacteria that survive and reproduce until eventually the entire population is resistant to the antibiotic.

"Maybe, if in earlier decades medical professionals had consulted with evolutionary biologists about the long-term consequences of different antibiotic treatment approaches, we wouldn't be in the scary conundrum we have today, with lots of multiresistant bugs around."

On to the next generation

After completing his postdoctoral work in Indiana, Denver turned his attention away from *C. elegans* for a year to take a position as a genomics instructor and researcher at Massey University in New Zealand, where he examined mutations in an isolated population of Antarctic penguins. Scientists had collected and carbon dated generations of penguin bones that had frozen in the Antarctic ice. Denver then cracked them open and sequenced their DNA, creating a timeline of DNA mutation that stretched back 45,000 years and another opportunity to examine how fast mutation occurs.

Along with this ongoing research, Denver, who is now an assistant professor of zoology at Oregon State University, continues his work with *C. elegans*. He's working to repeat his experiment with other species of nematodes and learn more about exactly how natural selection affects the rate of mutation. In 2007, scientists in Edinburgh, Scotland, replicated his *C. elegans* experiment using fruit flies and came up with results that corroborated Denver's findings.

Sitting in his office wearing a T-shirt and a Mizzou ball cap, Denver remains modest about his work. But like a child watching critters squiggle out from under rocks, he still marvels at the complexity of the world beneath our feet and the significance of one tiny worm.

"Soil is the most complicated ecosystem on earth, and just a spoonful of it will have anywhere from 1,000 to 10,000 nematodes and hundreds of different species of them," Denver says. "By studying just one of these worms at the most fundamental level, we can get to the real root of a huge problem and understand the base of a tree that affects everything. We could cure cancer by looking at something we dug up from the ground — that's just cool."

About the author: Sona Pai, BJ, BA '99, is a freelance writer in Portland, Ore. Her essay "Mangoes, Memories — and Motorcycles" is included in the anthology Best Food Writing 2008, *published by Da Capo Press.*