



by ROBIN MEJIA

Guided  
By His Inner  
Compass



*Nobel laureate Mario Capecchi has the  
confidence to march to his own drummer and  
the patience to focus on the long view.*

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*The mouse looks fine as Mario Capecchi holds it in his palm for the TV crew to shoot. You'd never know the animal has a condition analogous to human obsessive-compulsive disorder (OCD). In fact, you'd be more likely to think that maybe Capecchi does. As he describes the mouse's obsessive grooming habits, he mimics them for the camera, his hands doing half the explaining for him.*

The TV crew, from an Italian network, was in the San Francisco Bay area last fall filming a show on venture capital. When the 2007 Nobel Prize in Physiology or Medicine was announced, they detoured to the University of Utah to interview Italian-born Capecchi, an HHMI investigator who shared the prize with Oliver Smithies (at the University of North Carolina at Chapel Hill) and Sir Martin Evans (at Cardiff University in the United Kingdom) for developing techniques that enabled the creation of “knockout mice.”

The OCD mouse is just one example. After Capecchi and then-student Joy Greer knocked out a gene called *Hoxb8* in a mouse embryonic stem cell line, the mice derived from these cells engaged in compulsive grooming of themselves and neighbors. Though *Hox* genes are best known for regulating development, this experiment showed they could also control behaviors in adults.

When scientists want to understand what a gene does, one of the first things they do is create a mouse knockout. “I think it is the most powerful method we have for understanding the function of mammalian genes,” says Francis S. Collins, director of the National Human Genome Research Institute at the National Institutes of Health (NIH). Students today take for granted that knockout technology has always been around, he says.

### *Life after Harvard*

When Capecchi was an undergrad at Ohio's Antioch College, molecular biology was a new field; he studied physics and chemistry. However, after a couple of obligatory work-study semesters in biology labs at the Massachusetts Institute of Technology, Capecchi knew he wanted to be a molecular biologist. During an interview at Harvard University, he asked James Watson where he should go for graduate school. Watson told him “here,” and that's where Capecchi went.

“He has probably told you about Jim Watson's advice, ‘Don't waste time on small questions,’ which he took to heart, much better than most of us did,” says Ray Gesteland, a geneticist in Watson's lab at the same time and now a colleague at the University of Utah. “As a graduate student, Mario was clearly unique,” recalls Gesteland. “His experiments were always more elegant. They were designed better, and they worked better.”

When Capecchi was ready to leave Watson's lab, he got an offer from Harvard Medical School to stay and start one of his own. By the early 1970s, however, Capecchi was no longer happy at Harvard. “What they do there is hire a bunch of people all doing similar kinds of things and then watch Darwinian principles at play,” he says, observing that this created an incentive for scientists to work on short-term projects. Capecchi preferred the long term.

When the University of Utah came calling, promising him freedom to focus on big questions without having to justify his existence every couple of months, he had some qualms—one doesn't leave Harvard lightly. So, again, he asked Watson for advice. “He said you can do good work anywhere,” Capecchi recalls. With four students and their families, he packed up his lab and caravanned across the country.

### *Sticking to His Guns*

In 1977, Capecchi identified a long-term challenge for himself that ultimately assured his renown. He had read a paper by two Columbia University researchers, HHMI investigator Richard Axel and Michael Wigler, who made a solution of DNA with calcium phosphate; when they put it on top of a cell culture, the cells took up the DNA. Most of the time, cells' digestive enzymes destroyed it, but in about one cell in a million the DNA made it into the nucleus able to function.

Capecchi figured that a rate-limiting step in that experiment was technological—the mechanical process of getting the DNA into the nucleus—and that success would simply be a matter of innovation. “Technology itself is what makes things really jump,” he says. “All of a sudden you open up new ways of measuring things, new ways of seeing things. It's those jumps that make the significant breakthroughs in science.”

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MARIO CAPECCHI *The ability to concentrate on a chosen topic for long periods of time at the exclusion of everything else, he says, is one of his strengths.*

At the time, a colleague in the lab next door was doing electrophysiology with the aid of a setup that Capecchi says looked like it could be fashioned into an extremely fine hypodermic needle. He adapted the setup to create such a needle, attached DNA containing a selectable gene to a tiny fragment of viral DNA—an enhancer, though no one yet knew what it was—and then used the needle to shoot this complex into the cell nucleus. “That worked enormously efficiently,” he recalls. “About one in three cells actually picked up the DNA in functional form, so it was about a million-fold improvement in transfer of functional DNA.”

He and his students then teased out the way cells incorporated the DNA into their nuclei and found a surprise: sometimes the cells used “homologous recombination”—a physical rearrangement of genetic material between two strands of DNA—to stitch together multiple copies of the same DNA stretches one after the other. This observation proved that mammalian cells had the machinery to enable homologous recombination between copies of injected DNA molecules.

It was a small leap in imagination to envision that the same machinery could be made to bring about an exchange between a

chosen piece of DNA and the similar sequence resident in the genome of the mammalian cell. In 1980, Capecchi submitted a grant proposal to the NIH for three projects, one of which would use the newly observed homologous recombination machinery to create such gene-targeting events.

“They essentially said ‘No good, drop it, not likely to succeed’ and also gave me one of the worst scores I’d ever received,” says Capecchi. He got the NIH funds, but, “the message was very clear: shelve gene targeting and put all your efforts into these other two projects. So I put all of our efforts into gene targeting!”

### *Something Big*

*Capecchi used his grant money exactly how the NIH had told him not to—a potentially career-ending move if the experiments failed. Fortunately, by the time he needed to renew the grant in 1984, he had succeeded in making gene targeting work in mammalian cells. The same year, Capecchi heard a talk, by a graduate student of British researcher Martin Evans, explaining how their lab had cultured embryonic stem cells from mouse embryos. Capecchi’s*

goal had always been to build a mouse with his targeted mutations, so he called Evans to ask if he and his wife, Laurie Fraser, could visit Evans' laboratory to learn how to work with these cells.

Meanwhile, Oliver Smithies had been working on the very problems Capecchi was pursuing, albeit with a different approach. While Capecchi wanted to induce mutations in mice, Smithies hoped to repair bad DNA with a view to gene therapy. Evans made his cells available to Smithies as well.

The situation was a recipe for a classic scientific rivalry. Yet what resulted was magnanimity, based on mutual respect and support. In an interview with the Nobel Foundation shortly after learning he'd won the prize, Smithies said, "I so much admire the work of Mario Capecchi and Martin Evans. So that's a big delight to me, to share it with them."

"There are many stories about two Nobel prize winners who won't talk to each other," says Capecchi. "That would be awful. In this case, all three of us actually are good friends. We were following our own pathways, but at the same time we tried to help each other."

That's not to say Capecchi was broadcasting his progress. In 1987, he published a paper on introducing targeted mutations; Smithies followed almost immediately with a study showing how he had repaired a faulty gene. In both cases, the research was based on mouse embryonic stem cells. Two years later, Capecchi published a description of his first knockout mouse.

### *To Hell and Back*

*As the camerawoman continues filming, Capecchi returns the OCD mouse to its cage and walks the Italian TV crew through the rest of his lab. A short man with waves of well-groomed, if still somewhat*

unruly, gray hair, he is dressed for the interview in a crisp tan shirt and dark silver paisley tie, his black clogs the only hint of his standard campus wardrobe. There are rows of benches and cold rooms where the 39 people who make up Capecchi's lab—researchers, technicians, and support personnel—continue working. Three weeks after the Nobel announcement, they seem numbed to the presence of reporters.

Back at his office, he settles into a gray desk chair, picks up his laptop, and brings up an impressionistic oil painting of two kids at an outdoor picnic table. "That's my mother and her brother," he explains, as the camerawoman zooms in. The artist, Capecchi's grandmother, raised her American children abroad. The next photo, a black and white, is of his uncle as an adult. Edward Ramberg was a physicist whose work helped lead to the invention of the electron microscope and television. "He wasn't very proud of the latter," Capecchi says. "TV wasn't allowed in his house." The focus then turns to a framed etching of his mother, also made by his grandmother, on the wall across from Capecchi's desk.

The TV correspondent asks if he has pictures from his own childhood. "Well, there aren't any from Italy," he explains. The reporter should have known this; Capecchi's triumph against a horrific childhood is one reason he's here.

Capecchi, the result of his mother's affair with an Italian Air Force officer, was a child during World War II. One of his first memories dates from age three and a half, when his mother, a vocal opponent of the fascist government, was arrested at their home. Having seen the arrest coming, she had prearranged for a local peasant family to take him in, leaving them with money for his care. When the money ran out a year later, the boy was left to fend for himself. His father took him in a couple of times, but

#### KNOCKOUTS ON THE FAST TRACK

*Although a couple of thousand mouse knockouts have been described in scientific papers, fewer than 1,000 are available at repositories such as the Jackson Laboratory in Maine, primarily because of the expense of archiving and distributing them.*

Because knockouts have become so essential to medical research, an approach to improve accessibility is needed, says Francis S. Collins, director of the National

Human Genome Research Institute at the National Institutes of Health (NIH). As a result, the NIH, European Union, and Genome Canada are funding efforts to create a public library of mouse embryonic stem cells with knockouts of each of the more than 20,000 protein-coding genes—and to do it within the next four years.

Scientists who engineer a new knockout mouse from embryonic stem cells obtained

from the library will be required to send back a frozen sperm sample of their creation. By eliminating much of the up front work, the hope is that the project will encourage researchers to create mouse models of rare diseases.

Collins says that this effort is perhaps the best reflection of the importance of the work of Mario Capecchi, Martin Evans, and Oliver Smithies. —R.M.





# “FOR A SCIENTIST, contradictions ARE OFTEN A POINT OF INTEREST.” —Mario Capecchi



never for long, a few weeks at most. So at age four and a half he learned how to live on the streets—stealing food, fighting, occasionally ending up in orphanages plotting his escape, and, most of the time, hungry.

His mother was imprisoned in Germany for the duration of the war. On Capecchi's ninth birthday, she found him at a hospital in the town of Reggio Emilia, stripped of his clothes so that he couldn't escape. He had been admitted for typhoid and malnutrition, a condition that was failing to improve on the hospital's daily rations of chicory coffee and a piece of bread.

His uncle, who lived in Pennsylvania, sent boat tickets, and within a couple of weeks the mother and son left for America. That's Capecchi's memory of events. But one of the side effects of his Nobel win is that a pair of AP reporters went to Italy to document the details of his story—something that Capecchi, who says he “closed the door” on that part of his life when he arrived in the United States, had never done. He didn't tell anyone about his wartime experiences—not even, until about 12 years ago, his wife, Laurie.

The AP team uncovered documents in Italy and Germany that fit with many of Capecchi's memories and raised questions about others. For example, no records have been found of his mother's internment in Dachau, where his uncle thought she had been held. (She did not want to talk about her wartime experience.) Capecchi says he would eventually like to do more research on his early life, such as examining records from other concentration camps, and appreciates the records that reporters have turned up. “For a scientist, contradictions are often a point of interest,” he says.

The reason Capecchi decided to finally discuss his childhood remains unchanged. When he received the Kyoto Prize in 1996 and was asked to provide an autobiographical statement, he says he hoped that by opening his “Pandora's box,” he could communicate that early deprivation doesn't necessarily affect an individual's potential. Any child, given the chance, can amount to something.

## *Taking the Long View*

*Capecchi is thoughtful, mild-mannered, and gracious, focusing completely on whomever he is with. He laughs easily and seems comfortable in his own skin. He is circumspect about his family, however. When he talks about his daughter, now in college in California, it's only to say that he hopes she'll be able to find work*

she loves and is passionate about, regardless of whether it's in science, art, or something else.

Capecchi has certainly found work that *he* loves, and he confronts it with ambition, as well as patience. His publication record, while truly impressive, has noticeable slowdowns. Those gaps don't reflect slowdowns in his work, he observes, but rather a willingness to wait until he has something substantial to say.

Capecchi has had plenty to say about the many knockout mice his lab has contributed. He and his team have pushed the technology to create mice with multiple genes knocked out and also what he calls conditional knockouts—mice in which he can turn a gene off, at will, in a specific tissue or phase of development.

While a four-year international project is attempting to speed the development of knockout mice using the techniques his lab pioneered (see sidebar), Capecchi is moving on. Once again he is taking the long view. “This next project we're working on is probably 20 years,” he says.

He elaborates, noting that most mammals share the vast majority of their genomes; mammalian bodies are all based on the same set of constituent parts. Mice have tiny paws and bats have huge wingspans, but they're both created from the same set of components. Capecchi explains that many evolutionary changes appear to be additive; they happen because members of a given species acquired new, usually added, characteristics as a consequence of random genome modifications or mutations that provided them with a selective advantage.

If, for example, he were to take a set of genes from a bat, add them to the DNA of a mouse embryonic stem cell, and then generate mice from these cells, he may be able to observe changes in the mouse that reflect what the added genes were doing. If the mouse fingers grew abnormally long, he'd know the added genes were important for controlling digit length.

The logic is elegant, but there are many reasons to judge that the experiment might not work. Normally, only tiny fragments of exogenous DNA are introduced into a cell nucleus, yet Capecchi is talking about adding very large, defined pieces of DNA encompassing a significant portion of a chosen chromosome. Even if he does get the DNA in there, he'll still have to successfully generate a mouse. Multiple copies of a given gene can be fatal to embryos. Indeed, not long before he won the Nobel, the NIH rejected a grant proposal that outlined this work.

This HHMI investigator is going ahead anyway. The high risk, in Capecchi's eyes, is worth the potential payoff. ■