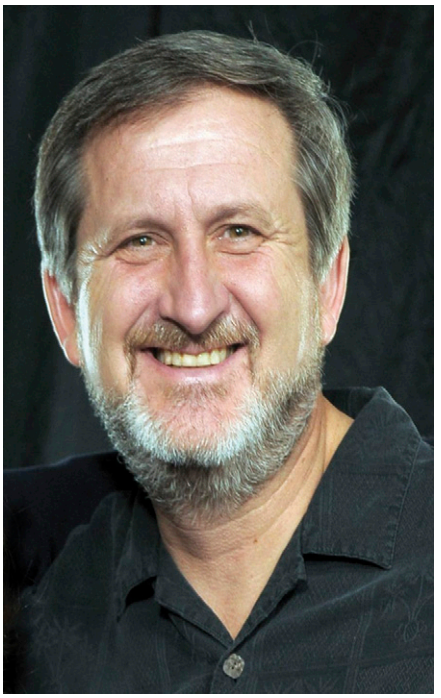
Profile of David M. Hillis

Philippe Padieu was called a modern-day Casanova. Women said they were drawn to him for his sweet personality and charm. However, Padieu harbored a secret. In 2009, he was found guilty of aggravated assault for purposefully infecting six women with HIV and sentenced to 45 years in prison. Credit for Padieu's guilty verdict goes partly to David Hillis, an evolutionary biologist at the University of Texas at Austin.

Hillis, elected to the National Academy of Sciences in 2008, has devoted his research career to phylogeny, the study of evolutionary relationships. His work tackles some of the greatest questions of evolutionary biology: How do species arise? How do genes diversify and acquire new functions? How do pathogens evolve, and how can that information be used to understand diseases? And ultimately, can we reconstruct the complete Tree of Life and use that information to help make predictions about biology?

Before the Padieu case, Hillis and colleagues had used phylogeny to determine the degree of relatedness among HIV carriers. However, in the Padieu case, prosecutors needed to show that the HIV traveled from Padieu to his victims, rather than the other way around. HIV was known to infect each host with billions of virions. However, when the virus travels from a source to recipient, a genetic bottleneck occurs and typically only one of those virions makes the jump into the new host. Because the virus evolves rapidly, Hillis' team was able to show that six of the samples were derived from the seventh. In court, that seventh sample was revealed as belonging to Padieu (1).



David M. Hillis.

Amphibian Evolution

Much of Hillis' early exposure to science came through his father, an Air Force physician and epidemiologist. Hillis was born in Copenhagen, Denmark, but his family later relocated to San Antonio, TX. There, in the early 1960s, Hillis' father treated a veterinarian who had contracted hepatitis from one of the chimpanzees used in the US space program. That case and other ones like it overturned the belief that chimps were immune to the disease, suggesting instead that chimps could be carriers. Understanding hepatitis' mode of transmission consumed the elder Hillis' subsequent work, and the family relocated to the Congo so he could study chimpanzees in the wild. En route to Africa, though, the Hillis' belongings were stolen, a strangely serendipitous experience. "We had no toys. There were no distractions like television or anything like that. The primary way I had to entertain myself was to run around the jungles of Africa and catch lizards and snakes and butterflies," Hillis says.

That scientific curiosity stayed with Hillis as the family fled the Congolese civil war. The family later lived in Calcutta, India, and then Baltimore, MD, where both Hillis' parents took up faculty positions at The Johns Hopkins University. By high school, Hillis had grown intrigued by reptiles and amphibians. "I became fascinated with reptiles in Africa, where I'd caught and kept lizards and learned that many adults were scared of snakes," he recalls. "A little kid with a snake feels powerful, I think, as many adults have learned an unnatural fear of them." As an undergraduate, Hillis realized that amphibians were also ideal organisms for studying how species diverge and evolve. He graduated in 1980 with a degree in biology from Baylor University in Waco, TX, and entered a graduate biology program at the University of Kansas in Lawrence to study amphibian evolution.

At that time, phylogenies were largely constructed based on the morphology of organisms, by using such characteristics as shape and size. However, Hillis realized that much more information on evolutionary relationships was encoded in genomes. Researchers were just beginning to analyze chloroplast and DNA, passed down through the maternal line. However, both types of analyses had their shortcomings: chloroplast DNA analysis was restricted to plants, and mtDNA evolves so quickly that it was best suited for reconstructing relatively recent divergence events. So Hillis looked to nuclear ribosomal DNA, as well as nuclear-encoded proteins, to reconstruct the evolutionary history of amphibians. The results revealed enormous hidden diversity among morphologically similar amphibians (2, 3). Rather than throwing out centuries of morphological work, however, Hillis worked to integrate molecular and morphological studies of phylogeny (4).

Soon Hillis turned his attention to how individual genes evolve and diversify. For instance, when Hillis and his colleagues studied the evolution of the *ZFY* gene, then thought to trigger male development in mammals, they found that the gene was unrelated to sex determination in reptiles (5). **"**It was one of the first indications that the *ZFY* gene was not actually the sex determination gene, as it was initially thought to be," Hillis says. Soon after, the actual sex-determining gene of mammals, *SRY*, was discovered.

Hillis' work in frogs and reptiles made clear that phylogenies could reveal how life on Earth evolved. Rather than look at single species, Hillis now devotes much of his time to modeling evolution on computers and coming up with ways to detect evolutionary patterns in enormous datasets. Although his early interest in science may have come from his father, Hillis gives credit for his more recent work to his mother, a biostatistician.

Watching Evolution

Hillis' shift toward statistical and computational approaches to evolution began when he accepted a faculty position at the University of Texas at Austin, where he now works. There, he met his colleague Jim Bull, initially a skeptic of phylogenetic analysis. "He didn't think there were any convincing studies of the accuracy of phylogenetic methods," Hillis says. Bull had reason to doubt. By that point in the late 1980s, phylogenetic analysis was growing in popularity, but its accuracy remained in question. Because much evolution occurs over lengthy time scales, it usually cannot be observed directly. Morphological change can be examined in the fossil record, but molecular

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changes—increasingly the basis for phylogenetic studies—are not preserved in fossils.

Hillis was convinced he could get Bull to come around. So they made a deal. "The stakes were high," Hillis jokes. "We bet a quarter." For a convincing case, Hillis and Bull needed to find a species that evolved fast enough to be observable in real time—one that could grow, reproduce, and accumulate mutations in minutes rather than millennia. The two researchers settled on T7 bacteriophage, a virus that doubles its population in less than 15 minutes. They started with a single ancestral stock and let it reproduce for hundreds of generations, separating out colonies at predetermined intervals. To encourage rapid evolution, the progeny were exposed to a chemical that promotes mutations during viral replication (6).

Hillis, who was blinded to the experimental setup to avoid biasing the results, mapped the genomes of the evolved experimental viruses and used five phylogenetic methods to reconstruct the phage's evolutionary history. When the blinded labels were decoded, Bull agreed that the estimated trees provided an accurate picture of the actual evolutionary history. Moreover, the ancestral genomes at each branching point in the phylogeny had been reconstructed with greater than 98% accuracy. The pair soon devised ways to test the validity of the phylogenetic methods under more difficult conditions, such as subjecting divergent lineages to vastly different rates of evolution or similar selective conditions (7, 8). Even then, the phylogenetic methods could account for the evolutionary changes.

That meant that phylogenetic methods could help researchers piece together evolutionary history, including the detailed reconstruction of ancient gene sequences. "A lot of people have extended that work to look at the gene sequences of extinct organisms extending back to the common ancestor of life," Hillis says.

Looking back to the dawn of life, although, presents other challenges. Unlike the experimental viral trees, the evolutionary relationships in most phylogenetic trees cannot be observed directly. So how can biologists assess the accuracy of ancient phylogenies? In a series of pioneering papers, Hillis and his colleagues showed how statistical resampling approaches and Monte Carlo simulations, which predict the probabilities of certain outcomes based on prespecified models, can be used to assess confidence in a given phylogenetic tree. The researchers tested the reliability of these statistical approaches on experiments with simulated phylogenies as well as the bacteriophages they had studied in the laboratory (9, 10).

In the mid-1990s, biologists began tackling phylogenies with hundreds of species. Many people were skeptical that such large phylogenies—which Hillis says contain more potential solutions than the number of fundamental atomic particles in the universe—could be reconstructed accurately. One of the larger trees at the time was produced by Pam and Doug Soltis, evolutionary biologists who now work at the University of Florida in Gainesville. The duo had reconstructed the evolutionary relationships among 228 species of flowering plants by comparing their ribosomal RNA genes (11). However, some researchers were unconvinced by the Soltises' findings, arguing that efforts to recreate the evolutionary histories of just a handful of species required the analysis of many more genes—and that the same principle should hold true for larger data sets, like the flowering plants.

To resolve the dispute, Hillis simulated the evolution of the ribosomal RNA genes on a computer. Expecting to find that the Soltises needed more data, Hillis was surprised to discover that denser sampling of species generated more realistic phylogenetic trees. "Analyzing more genes was helpful, but analyzing more species was critical for phylogenetic accuracy," Hillis says. "It was great news for the Soltises and for the field as a whole" (12).

Densely sampled trees provided more accurate reconstructions of evolutionary history and allowed researchers to develop more detailed and accurate models of DNA evolution (13, 14). As a result, Hillis says, "many researchers are now tackling problems with thousands of species." Leading the charge is the National Science Foundation and its ambitious Tree of Life program, which aims to generate a phylogenetic tree connecting all life forms on Earth (15–17).

Estimates, however, suggest that the Earth contains approximately 9 million species, making it difficult to view the complete Tree of Life in 2D space, Hillis says. So Hillis and his colleagues developed new techniques to visualize complex phylogenetic information (18). Among other solutions, they designed a circular tree with 3,000 clickable points, each representing a different taxonomic group. Clicking a point opens up another 3,000 points, for a total of 9 million points—or species. "That way, the whole tree of life can be represented in two layers," Hillis says.

An early version of that circular tree of life has now entered pop culture: some of Hillis' graduate students have tattooed the design on their backs and a giant Tree of Life mural currently graces the entranceway of the biology building at University of Texas' Austin campus. Next to the location of humans, the tree has a "you are here" sign. "At the beginning of the semester, there are always a lot of mystified students looking at it saying 'I don't understand... is this a map of campus?'" Hillis says.

Evolution Matters

The theoretical groundwork laid, Hillis became interested in applying phylogenetic methods to answer questions about the molecular processes underlying evolution. Hillis first ventured into the study of HIV transmission following a high-profile case in the early 1990s in which an HIV-positive dentist in Florida was accused of transmitting the virus to several patients. After evaluating blood samples from infected individuals, the Centers for Disease Control determined that the HIV strains in the doctor and his patients were related. Hillis was asked to review the results of this new methodology. The methods the Centers for Disease Control used were sound, Hillis says, but the case never went to trial because the dentist died in the interim.

It took another high-profile case for the phylogenetic analysis of HIV to make its way into US courtrooms. In the late 1990s, a physician stood accused of intentionally injecting his ex-girlfriend with an HIV-infected blood sample from an unwitting patient. The prosecution thus needed to show that the HIV samples between the patient and ex-girlfriend were related (19).

The science behind showing the relatedness of HIV strains is straight out of the phylogeny textbook, Hillis says. As noted, when HIV passes from source to recipient, typically only one virion makes the jump. That virion then diversifies rapidly inside its new host. To determine relatedness between samples, researchers take many samples of the virus from the victim and defendant and reconstruct the phylogeny of the virus as it mutates and evolves into different strains. The resultant trees either support or rule out hypotheses about the virus's transmission from person to person.

In the case of the physician's girlfriend, her HIV sequences supported the hypothesis that she contracted HIV from the physician's patient. The final step was for the courts to determine whether to permit these HIV phylogenies as evidence in the courtroom. Hillis was called in as an expert witness to explain phylogenetic analysis to the court. The judge agreed to admit the phylogenetic evidence—together with all the other evidence about means, motive, and opportunity—and the jury found the physician guilty of attempted murder.

Hillis' Inaugural Article (1) pushes forensic applications of phylogeny a step further by showing how phylogenies can also provide information on the direction of transmission. Because of the genetic bottleneck that occurs at the point of transmission, a recipient's tree is expected to be nested within the source's tree, Hillis says. In the case of Phillipe Padieu, and a very similar case from Washington state, information about the direction of transmission was integral to the establishment of the men's guilt.

However, if the science behind phylogenetic analysis of HIV is straightforward, the ethics are not. Practiced incorrectly, phylogenetic analysis could become a way of criminalizing those with HIV, Hillis cautions. Fear of criminal liability could also prevent people from getting tested, which could be especially problematic in AIDS-ridden countries. Rather, Hillis says, before applying phylogenetic analysis to transmission questions, it is fundamental to first have evidence of criminal intent, as well as a clear a priori hypothesis to test.

Science Education

Hillis' latest endeavor may turn out to be the most complicated. As with his research, Hillis says he takes his "role as an educator very seriously." He penned his first textbook, a how-to manual for reconstructing phylogenies, in 1990 (20–23). More recently, Hillis has become an outspoken critic of efforts to weaken the teaching of evolution in schools, a particularly heated debate in Hillis' home state of Texas. Part of informing students about the importance of evolution, Hillis says, is making biology more accessible and relevant to students. "The contents of college textbooks generally are accurate," he says, "but I find it depressing how boring most of them are."

In the past few years, Hillis has authored two introductory biology textbooks, *Life: The Science of Biology*, and *Principles of Life*, to address that shortcoming (24–26). Thanks to that work and his efforts to advise the Texas State Board of Education on the science curriculum, Hillis won the Stand Up For Science award in 2009 (from the Texas Freedom Network) and the Friend of Darwin award in 2010 (from the National Center for Science Education).

Hillis applies and enjoys evolution on a personal level as well. The proud owner of the aptly named Double Helix Ranch in Texas, Hillis has been studying his population of Texas Longhorn cattle to understand their evolutionary roots. According to historic accounts, Christopher Columbus brought cattle from Europe to the Caribbean in the late 1400s. From there, the cattle were taken to Mexico and the American Southwest, where they escaped and formed feral herds. Over the centuries, the cattle evolved longer horns, which served as defense against predators. With the cattle genome now complete, Hillis and his students are comparing breeds of domestic cattle from region to region to reconstruct their population histories.

As with his other work, Hillis' research into the cattle has shifted from mere scientific curiosity to a search for practical applications. Texas Longhorns have many genes that confer disease resistance, fecundity, longevity, and ease of birthing, all of which are important in creating hardy breeds of grass-fed, free-range cattle, he says.

Evolution, says Hillis, helps answer both sweeping questions (what is the origin of life?) and ordinary ones (how does one create better cows?). Hillis' motivation for studying evolution is simple: "I like thinking about the common ancestors that I share with the cotton fibers in my shirt," he says.

Sujata Gupta, *Freelance Science Writer*

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