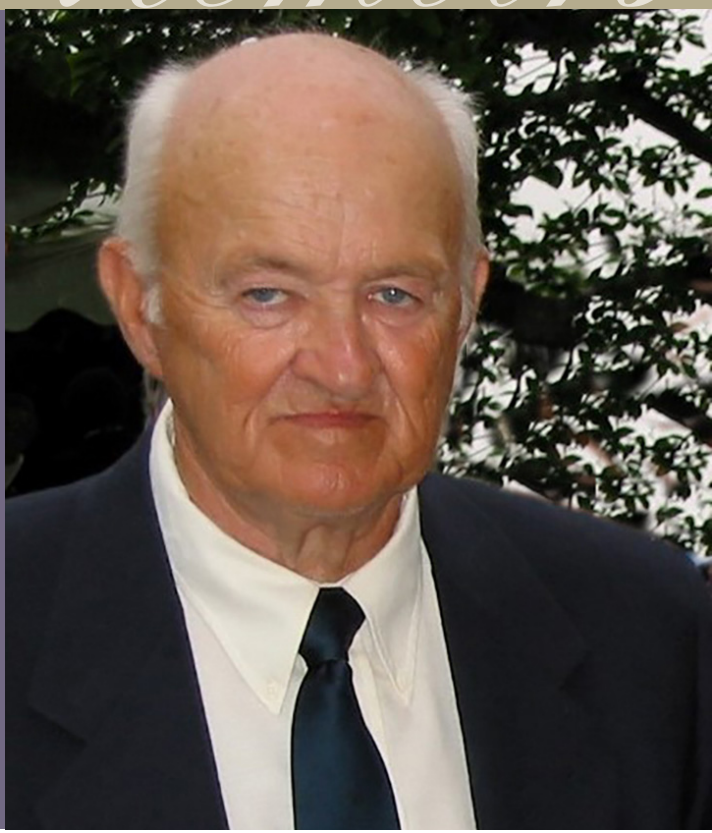
Charles S. Levings III

1930–2017

BIOGRAPHICAL *Memoirs*



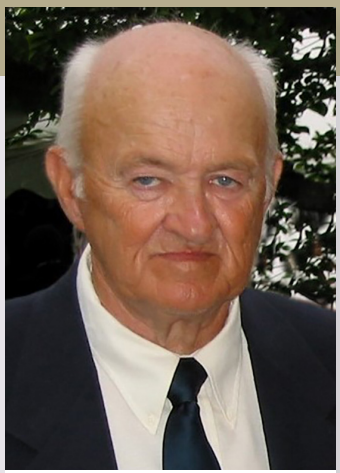
A Biographical Memoir by
Ronald Sederoff
and Brian Larkins

NATIONAL ACADEMY OF SCIENCES

CHARLES SANDFORD **LEVINGS III**

December 1, 1930–January 18, 2017

Elected to the NAS, 1987



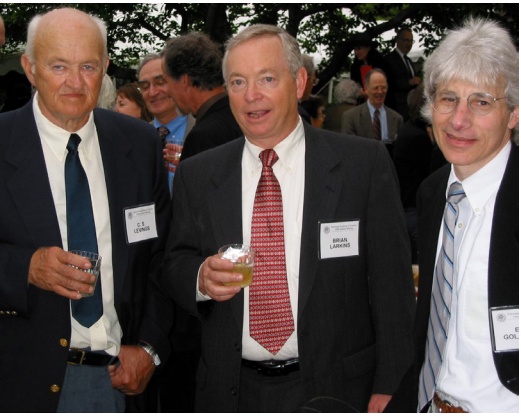
Photography by Brian Larkins

By Ronald Sederoff and Brian Larkins

Charles Sandford Levings III was an exceptionally talented scientist who had a major impact on plant molecular biology through his pioneering studies of maize mitochondrial DNA. He was best known for a series of elegant experiments[1] that led to the discovery of a novel single maize gene responsible for the "Texas" type of cytoplasmic male sterility (*cmsT*) and the plant's susceptibility to Southern corn leaf blight, a devastating fungal disease that decimated the hybrid corn seed crop in 1970 and 1971.[2] Plant mitochondria are discrete organelles with a genome that represents about 0.1 percent of the DNA found in a typical plant nucleus. Levings was able to identify features of the mitochondrial genome that are unique to plants, as well as those features common to plants and animals. The maize mitochondrial genome was viewed as a small version of the much larger plant nuclear genome, and it served as a technical and conceptual predecessor to the genomic revolution that was yet to come. Levings' early applications of genomic technology led to mitochondrial gene discovery, gene cloning, sequencing, and proteomics, all focused on the nature of maize cytoplasmic male sterility, a problem of great value to the corn hybrid seed industry because of the association of *cmsT* and the Southern corn leaf blight.

Levings attended Depauw University from 1947 to 1950, at which time he entered the army and served in the Korean War. After his return he earned B.S. (1953), M.S. (1956), and Ph.D. (1963) degrees in agronomy from the University of Illinois. Upon receiving his doctorate, he took a position as a postdoc in quantitative genetics at North Carolina State University, where he taught and did his groundbreaking research for the next 34 years.

Charles Sandford Levings III—Sam, as he was called by friends and colleagues—was born on December 1, 1930, in Madison, Wisconsin, and grew up in the small town of Paris, Illinois. He joked that the town was so small that he and his friends would watch



Sam, Brian Larkins, and Bob Goldberg at NAS Garden Party 2001
(Photo credit Brian Larkins.)

haircuts for Saturday night entertainment. He graduated from Paris High School in 1947 and attended DePauw University in Indiana until 1950. He then served in the U.S. Army in the Korean War, where he was selected on arrival for the military police. Upon returning from the war, he proposed marriage to Mary Catherine Carroll, who was his spouse for the next 63 years. Enrolling at the University of Illinois at Urbana-Champaign, he received a Bachelor of Science (1953), a Master's of Science (1956) and a Doctor of Philosophy in Agronomy (1963). The subject of his dissertation was "An evaluation of the magnitude and effects of double reduction in autotetraploid maize." under the guidance of Professor D. E. Alexander.

Sam was then hired at North Carolina State University as a postdoc in quantitative genetics and subsequently began his 34-year service there in the Department of Genetics. Early in his career Sam defined a gene that controls the biosynthesis of dihydroxylflavones, which determine the browning of maturing maize silks. He was attracted to the emerging area of plant molecular biology. Because of the colorful variation of maize kernels, he saw promise in studies of the metabolic pathways of maize flavonoids, a subject that had been extensively investigated using traditional maize genetics. Much less research had been carried out on flavonoids at the biochemical level. His efforts did not proceed well, in large part because the metabolomic technology needed to complement the traditional genetics had not yet been developed. During a Christmas holiday, he decided to stop work on flavonoids and tossed out all of his cultures on a single day.

Sam then turned his attention to the nature of cytoplasmic male sterility (*cms*) in maize. *Cms* was used by the seed corn industry in the commercial production of maize hybrids[3] to replace the tedious removal of the pollen-producing tassels. Hybrid maize seed is generated from crosses of genetically diverse parental lines, and it produces larger, more vigorous plants with ears containing larger numbers of kernels. Self-pollination produces non-hybrid (inbred) seed, resulting in reduced yield and quality. The discovery of *cms* in

maize by Marcus Rhoades[4] was a great advance for the maize seed industry. Male sterile plants produce no pollen but are excellent female parents as a source of viable seeds. *Cms* has strict maternal inheritance and is genetically stable and predictable, greatly facilitating the production of hybrid corn seed. One source of *cms*, from Texas, known as *cmsT*, was planted widely and was estimated to produce 85 percent of the hybrid corn in the USA in 1970.[2]

That same year, there was a catastrophic epidemic of Southern corn leaf blight. The disease was caused by a new race (race T) of a pathogenic fungus, *Bipolaris maydis*, which ravaged U.S. corn fields. The losses in some areas of the country reached 100 percent of the maize crop. The economic losses were estimated at as much as a billion dollars (at present rates) and served as a dramatic object lesson on the hazards of monoculture. The virulence of the raceT pathogen was due to specific fungal toxins that affected the *cmsT* cytoplasm. Genetic and cytological evidence implicated the mitochondria as the target of the T toxin.[4] Therefore, Sam and his colleagues focused their attention on the organellar DNAs of maize *cms* to investigate the molecular basis of the sterility and toxicity of *cmsT* cytoplasm.

Sam turned to the newly discovered DNA restriction enzymes to compare mitochondrial (mtDNA) and chloroplast DNA (ctDNA) from different maize cytoplasms. The fragment patterns of chloroplast DNAs (ctDNAs) were essentially monomorphic, but the mt DNAs from different cytoplasms showed highly variable restriction fragment length polymorphisms (RFLPs). Each type of *cms* had a distinctive fragment pattern, but the discovery of the basis of male sterility and the target of the T toxin had to wait for the development of new genetic technology, the cloning and sequencing of DNA.

At the time of the first cloning of DNA from microbes and animals, cloning of plant DNA had not been successful, leading to a rumor that plant DNA could not be cloned. The failures can more likely be attributed to the difficulty of obtaining DNA of sufficient quality, because impurities would inhibit the enzyme activity needed for restriction, ligation, and DNA transformation. Organellar DNAs were the first successful targets for cloning plant DNA, most likely because of their higher purity and low complexity. Sam's research was at the forefront of the identification of the structure, function, and evolution of plant mitochondrial genes.

The mitochondrial genome of plants was quite different in organization, size, complexity, and the extent of sequence variation from the mtDNA of animals. Animal mtDNA was relatively small and fixed in size (about 16.5kb), and the major variation in mtDNA was

due to base substitutions. Plant mtDNA genomes were much larger and highly variable in size (200 to 2000 kb), due to repeated sequences and rearrangements created by recombination. Homologous recombination between large repeated sequences creates a dynamic mixture of DNA structures. One large DNA fragment from *cmsT* mitochondria was selected for further study, because it was specific to the *cmsT* cytoplasm and had unique and abundant transcripts. This fragment encoded a chimeric transcript containing portions of a mitochondrial 26S ribosomal gene, the ATPase subunit 6 gene and the tRNA-Arg gene. Two open reading frames were identified, one encoding a putative peptide of 13kD, (called URF13) specific to the T cytoplasm. These results led to the hypothesis that the URF13 protein was the cause of T-toxin susceptibility and the basis of *cmsT*. In a series of elegant experiments, Sam and his colleagues demonstrated that both of these hypotheses were correct.[5, 6, 7]

Because of its commercial value, hybrid maize breeders were prepared to carry out a large-scale effort to "break" the linkage of T-type male sterility and T-toxin sensitivity of the T-cytoplasm. But because Levings' research demonstrated that sterility and the toxin sensitivity were properties of the same URF13 protein, it was therefore highly unlikely that sterility and the toxin sensitivity could be separated by recombination even in a very large-scale cross. Consequently, these crosses were not carried out. Major Goodman (NC State University) estimates the savings for the corn seed industry at many millions of dollars.

Sam was a "short sleeper." A few percent of the human population are short sleepers and have a rare allele of a gene called ADRB1. [8] People with this syndrome need much less sleep than average, typically four hours or less per night. Some short sleepers are healthy, energetic, optimistic and successful. Sam worked hard and kept long hours. He was the first one in the lab in the morning and the last one to leave in the evening. In the early morning hours, he would hang out in all night coffee shops, with large yellow note pads and manuscripts, so he could think and write about his work. To have uninterrupted time during the workday, he also had quiet "hiding places" around or near the campus, where he could sit with his note pads to read, write and think in peace. Sam would write slowly, in very large script on his note pads. Once he had written a sentence or a paragraph for a manuscript or a grant proposal, it was finished and did not have to be revised. In contrast, many scientists, rewrite and revise many times. Sam was a writer, not a rewriter. One day he complained that he spent so much of his time reading and writing, rather than working in the lab. "I should have been an English major" he said. Sam's humor could be intense and biting, although at times unintentional. During one genetics

class where he presumed the students would all be familiar with meiotic cell division, Sam began his lecture by asking the class "I assume you have all had sex."

As a quantitative geneticist, Sam was not trained in biochemistry. But he had a tenacious approach to lab technology. If he decided that he needed to learn a new technique, he approached it like a bulldog. He sank his teeth into the methodology, and he kept "shaking it" until he made it work. Sam was the intellectual focal point of a small group of geneticists that met for lunch several times a week. The composition of the group (called the Lunch Bunch) varied from day to day, but it was a valuable forum for discussion of new and old science and scientific gossip. The group held to a high standard of scientific and intellectual integrity. Sam built an intellectual community that influenced the Genetics Department and the College of Agriculture and Life Sciences at North Carolina State. He influenced maize genetics, mitochondrial DNA studies, plant molecular biology, plant genomics, and the merging of plant breeding with quantitative and molecular genetics.

Sam was a natural leader whose Lunch Bunch was strong in both quantitative and molecular genetics, integrating fundamental and applied science, an approach that became the paradigm for plant biotechnology and genomics. His scientific leadership attracted the first AgBiotech company (Ciba-Geigy/Syngenta) to Research Triangle Park (RTP)—an area encompassing the campuses of NC State, UNC, and Duke—in 1983. Ciba-Geigy in turn became the nucleus for the development of RTP as an important AgBiotech industrial center. Major additions include BASF, Monsanto, Syngenta, and Novozymes. An estimated 100 startup companies have joined the AgTech sector at RTP. The Department of Agriculture established on the NC State campus an expanded unit of its Biotechnology Regulatory Services, which is responsible for safe introduction of genetically modified organisms for agricultural use.

In 1983 Sam was designated a William Neal Reynolds Distinguished Professor, one of the university's highest honors, which was created to recognize outstanding faculty in agriculture and life sciences. In 1987 he was both elected to the National Academy of Sciences and chosen to receive the NCSU Alumni Association Outstanding Research Award. In 1988 he was named a Distinguished University Professor and in 1990 was elected a Fellow of the American Association for the Advancement of Science (AAAS).

During his retirement, Sam spent time with his family and friends at his summer home at Topsail Beach, North Carolina, where he was well known by the fishing community at a local pier. In his early years, Sam was an avid outdoorsman who enjoyed gardening,

bird hunting, fishing, and riding a motorcycle. He will be remembered for his ever-present sense of humor. He was intolerant of bureaucracy, possessed a strong dose of common sense, was an advocate of social responsibility, and was dedicated to his family.

Sam's research describing the mechanism by which race-T of *Bipolaris maydis* created cytoplasmic male sterility in maize contributed to a paradigm shift in the way crop science was investigated. Previously, genetics and biochemistry were routinely applied to study the basis of the phenotypic variation associated with differences in crop performance. But Sam was among the first plant scientists to apply the molecular genetics and genomics approaches that would revolutionize academic and corporate agricultural research. Ultimately, this led to a world-wide enterprise in agricultural biotechnology that accelerated improvements in crop breeding and the creation and production of genetically engineered crops manifesting resistance to insects and herbicides. As a member of Section 62 (Plant, Soil and Microbial Sciences) of the National Academy of Sciences, Sam supported the election of many young scientists applying these approaches, and in so doing left a lasting imprint on the membership of Section 62 and agricultural research in general.

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