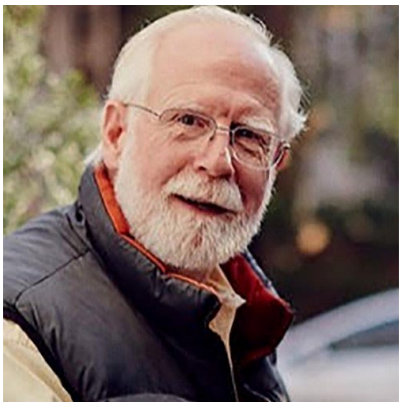
ASPB Pioneer Member

William Thompson



Thanks to an astute graduate advisor, I more-or-less backed into a career in plant molecular biology just as the field was beginning to take off. Partly because I started when there were few competitors, I was able to explore many different research directions. That freedom is less common now, given the modern emphasis on specialization. However, I've very much enjoyed allowing my research to evolve in multiple directions throughout a career that now spans 60 years from the time I declared myself a biology major. This narrative is a short history of that career and the people who shaped its evolution; I'm grateful to all of them.

During my formative years, I was often outside in the woods and mountains. Climbing mountains and seeing different plants at different elevations made me want to know more about why and how this happened. So my first ambition was to be a plant ecologist. Over the years, however, I became increasingly fascinated with the inner workings of plants. By the time I was applying for graduate school, I had decided to become a plant physiologist.

Along the way, I took classes and did an undergraduate thesis with Bill Jacobs, the plant physiologist at Princeton. After my junior year, I had extracurricular reasons for wanting to spend the summer in Seattle - I had family there and it was near some really great mountains - so Bill pointed me to Bob Cleland at the University of Washington (UW). I got a summer job as a part-time lab tech and learned that Bob and I got along famously.

Grad School. When the time came to choose a graduate school, the main contenders were Duke and UW. Duke was more prestigious, but I chose the known quantity, and I think I chose well. Not only was Bob a great advisor, but he was also the first person to suggest I work on molecular biology. I never looked back.

This was in 1966 when molecular techniques were just beginning to be applied to plants. Much of what was being done would be considered "bucket biochemistry" today. Volumes were usually measured in milliliters, not microliters. Pipettemen had yet to be born. If smaller volumes were needed, micropipettes were expensive little glass things that had to be cleaned with concentrated sulfuric acid and potassium dichromate. Microfuge tubes didn't exist. Virtually nothing was disposable.

Bob's first suggestion for a thesis project was that I should use the newly developed technique of molecular hybridization to look for mRNAs associated with floral induction in *Pharbitis nil* (Japanese morning glory). *Pharbitis* (also known as *Ipomea*), is a model short-day plant in which floral induction occurs during a single long night. So, the idea was to take multiple samples during that long night to extract and analyze RNA. Conceptually, this was a great idea. In practice, there were major difficulties. One was that we now know the analysis technique then available (hybridizing RNA to filter-bound total plant DNA) is quite insensitive to low-abundance sequences like most mRNAs.

Another difficulty was more profound. The experimental design required me to sleep on an air mattress in my student office, getting up every two hours to take samples. I realized this was a problem when I woke up sometime late in the night, found I'd missed two time points, and wondered where the alarm clock was that was meant to wake me up. I found it at the bottom of my sleeping bag - but had no memory of putting it there. Clearly, I wasn't cut out for that kind of experiment! Thankfully, Bob didn't seem to have a problem with me changing thesis topics.

I don't think I could have had a better Ph.D. advisor than Bob Cleland. I cherish the memories of Bob popping into the lab in mid-afternoon and saying "How about some coffee." We would then walk across campus to the student union and have all sorts of interesting discussions – mostly, but not always, focused on science. These informal meetings often included other students or faculty, and

sometimes there were visitors from other departments or universities. I particularly remember one time when Gene Nester from the UW Microbiology Department came to discuss ways in which microbiologists might make contributions to plant biology. Bob and I (mainly Bob; my contribution was mostly youthful enthusiasm) encouraged him to investigate *Agrobacterium*. He and his colleagues Milt Gordon and Mary Dell Chilton later started the Seattle *Agrobacterium* group which was a major player in *Agrobacterium* research for many years thereafter.

In learning about molecular hybridization, I relied to a considerable extent on Arnie Bendich, who was then a grad student working with Nester and Gordon and had previously worked with Roy Britten. I think Arnie was the first person to do hybridization experiments with plant DNA.

Eventually, I settled into a thesis project that was still based on molecular hybridization, but now aimed at changes in RNA induced by plant hormones. This one didn't involve getting up in the middle of the night and was therefore more successful than the flowering project. Despite the insensitivity of the techniques then available, I did find some effects, we published a couple of papers, and I got my degree.

Along the way, I took a plant ecology course from Art Kruckeberg and did a review paper on phenotypic plasticity that led me to the work of Clausen, Keck, and Hiesey at the Carnegie Institution's Department of Plant Biology. This was my introduction to ecological genetics, which I found fascinating. Some years later it was an important component of my decision to take a job at Carnegie.

Anyone of draft age during the late 1960s will understand that there was much anxiety associated with the Vietnam War. People were protesting, some were going to jail, others to Canada, and demonstrators were actually killed in the Kent State shootings. My status as a student, plus my marital status, kept me out of the draft through most of my graduate training, but there was a period when I was classified 1A due to a misunderstanding between the university and my draft board. The confusion was cleared up fairly quickly - although at the time it seemed to take forever. I would have gone if called, and it would have changed my life. So it was an object lesson in how much a career can depend on accidents of fate.

Postdoc years. After graduate school, in what turned out to be a fateful decision, I went to Winslow Brigg's lab to do postdoctoral research. Winslow and Bob Cleland were friends from their days at Stanford and Berkeley (respectively). Shortly before I was ready to finish my PhD, Winslow accepted a professorship at Harvard. He and his family traveled from California to Massachusetts via a scenic route including Seattle, and Winslow stopped in for a day at Bob's lab. He and I sat and talked on lab stools and went to coffee with Bob. After going through a formal application process – and receiving an NSF fellowship – I was on my way to Harvard without ever having considered another lab. Once again, I made a choice based on knowing the personality of my future boss. And, once again, I think I made a good choice.

Winslow's lab was focused on phytochrome at the time, having just shown that the phytochrome protein was actually about twice as large as the protease-cleaved forms previously studied. I did some work on phytochrome and published a paper on it with others in the lab. But I spent most of the time teaching myself to do DNA reassociation kinetics ("Cot curves"), because I had become fascinated with the work of Roy Britten and Eric Davidson. They had recently published seminal papers on repetitive DNA in eukaryotes and its potential significance for gene regulation. I'm forever grateful to Winslow for allowing me the freedom to do that independent work. It was the touchstone for the rest of my career.

First "real" job. After two years of postdoctoral work (short by today's standards, but normal for the time) my fellowship ran out and I was on the job market. I interviewed at Wisconsin, Texas, and Minnesota. I don't remember much about what happened at Wisconsin, except getting a question or two from Folke Skoog after my seminar. I don't think I got an offer. I also didn't get an offer from Texas. A month or two after my Texas interview, I was told by a faculty member there that the problem was that I had a beard.

I did get an offer from Minnesota, where beards were more readily tolerated. I enthusiastically accepted that offer and was all set to go when the legislature cut the university's funding and my offer had to be rescinded. The future looked grim. Fortunately, however, it wasn't long before I got an interview, and ultimately an offer, from the University of Massachusetts at

Amherst.

I loved Amherst and the UMass Botany department. We bought a house, adopted two kids, and I thought I'd stay forever. To finance the house on a beginning assistant professor's salary we rented rooms. One of our renters was Dan Cosgrove, who also did an undergraduate thesis in my lab before going to Stanford and getting his PhD with Paul Green.

I was fortunate to get an NSF grant on my first try - probably because DNA work in plants was still very novel. Amazingly, the panel thought I'd underestimated the cost of isotopes and therefore gave me slightly more money than I requested. Nothing like that has happened since!

Among the things I bought with that first grant was an electronic calculator. They had just hit the market then. It cost over $100 in 1972; with inflation that would be approximately $700 today. I had to get special permission from NSF to buy it. But, functionally, it was roughly equivalent to what you can now buy in drug stores for $5 or so.

My first graduate student, Diana Stein, was the wife of the Department Head, Otto Stein. Things could have been pretty awkward, but Diana and Otto were cool about it and there was no trouble. Diana's project was her idea, based on previous experience in a fern lab. It was focused on evolutionary relationships in the fern genus *Osmunda*. We used DNA:DNA hybridization to compare the genomes of three species that had previously been studied morphologically and in the fossil record.

To do this we had to work out how to get decent DNA out of fern extracts that were almost black with tannins and other phenolic compounds. It turned out that gel filtration columns separated the DNA from the black stuff. We also needed to work out how to label the DNA *in vitro* because we were collecting the ferns in the field and couldn't feed them isotopes *in vivo*. At the time, that meant working with radioactive iodine, which required doing everything in the hood and getting regular thyroid scans.

I think we may have been the first people to isolate DNA from ferns, as well as the first to use it to address an evolutionary question. We found that "our" three species were approximately equally diverged from each other, despite one being morphologically quite distinct from the other two. That was sufficiently novel for papers in *Science* and the *Journal of Molecular Evolution*.

Carnegie. While I was at UMass, Winslow Briggs accepted a position as Director of the Carnegie Institution's Department of Plant Biology. One of his first acts was to invite me to interview for a job. Located on the Stanford University campus, this Carnegie department had been the home of Clausen, Keck, and Hiesey, whose pioneering work on ecological genetics I first admired as a graduate student. Their work was done mainly in the 1940s, and none of the three were still active in the 1970s. However, a younger colleague of theirs, Malcolm Nobs, was still working there, and ecological adaptations were being actively studied in groups led by Olle Bjorkman and Joe Berry.

So I jumped at the chance to connect with the work on ecological adaptation and moved to California only a little more than two years after I thought I would stay forever at UMass. Thinking, of course, that I would stay forever in California.

I didn't make as many connections to the adaptation work as I had hoped. But I did make one right away, which turned into Heather Belford's thesis project on relationships in *Atriplex*. Heather had moved with me from UMass after having "cut her teeth" by participating in some of the *Osmunda* work there. At Carnegie, Olle and Joe were studying *Atriplex* for reasons having to do with its adaptation to desert environments. Notably, classical taxonomy had split this genus into two subgenera, each of which contained both species with conventional C3 photosynthesis and species adapted to use the C4 photosynthesis pathway. The presence of both pathways in both subgenera was interpreted as showing that C4 photosynthesis evolved twice independently within a single genus. If true, that would have been of considerable significance for people interested in the origin(s) of the C4 pathway. However, our molecular data did not support the subgeneric distinction, so it was no longer necessary to postulate independent origins for C_4 photosynthesis in the genus.

Possibly the most important thing I did at Carnegie was to hire Mike Murray as a postdoc. He and I were an excellent scientific team and started a personal friendship that has endured to the present day. Almost every day,

we would have long, wide-ranging scientific discussions while sitting on the edges of the heavy concrete planter boxes flanking the stairs between the main building and the coffee pot in the seminar room.

One day, those normally rock-stable planter boxes seemed to wobble like rickety chairs, and we quickly realized we were having an earthquake. Luckily, it turned out not to be a big one, but we scurried to safety as fast as we could, thinking about the heavy ceramic tiles on the roof above us. Later on, it was decided (probably by Winslow) to install plexiglass barriers on the shelves in all the labs, to keep our chemicals from tumbling off in the event of a bigger quake. After I left Carnegie, a larger quake did happen, and my understanding is those plexiglass barriers did their job quite well. And the ceramic tiles on the roof stayed on.

Genome Organization. Our work at this time was moving from evolutionary studies to comparative studies of genome structure. We had been impressed by large volumes of data showing that eukaryotes – and especially plants - varied tremendously in nuclear DNA content, with no obvious relationship to organismic complexity.

One reasonable hypothesis was that this variation reflected different amounts of repetitive DNA. So Mike and I, with contributions at different times from Rick Cuellar, Jeff Palmer, Deb Peters, and Rich Preisler, compared the genome of the garden pea (*Pisum sativum*) with that of mung bean (*Vigna radiata*), another legume in the same subfamily. Haploid pea nuclei contain 4.5 pg of DNA, which is about 50% more than the human genome, while mung bean nuclei contain only around 0.5 pg.

We showed that repeated sequences - most of which we now know to derive from various families of transposons - constitute a very high percentage of the pea genome and are interspersed more or less everywhere. Over 97% of randomly sheared DNA fragments 1300 nt long contained at least one repeated region. Interestingly, a large majority of mRNA sequences (analyzed as cDNA copies) hybridized to genomic DNA with much less repeat interspersion than bulk DNA, highlighting structural differences between genic and non-genic regions of the genome.

In contrast, the mung bean genome had fewer repeats, a larger fraction of single-copy DNA, and much less repeat interspersion – comparable to the animal genomes being studied at the time. This result and related data led Mike and I to develop the hypothesis that genomes are shaped by cycles of amplification, deletion, and sequence divergence over evolutionary time. This idea is commonplace now, with lots of empirical data supporting it. But it was quite novel in 1981.

Technical Issues. When we began the comparative work, we were influenced by a new DNA isolation procedure in which tissue extracts were made in the presence of high concentrations of urea. This procedure seemed to work well, although we later discovered that DNA prepared this way exhibited several-fold faster reassociation kinetics than it should have. Even an added E. coli DNA tracer was accelerated. Something in our extracts was interfering with – accelerating – normal renaturation.

We never definitively identified this acceleration factor, although we could see it using Schlieren optics in an analytical ultracentrifuge and eliminate it by purifying DNA in preparative CsCl gradients. However, we felt it was most likely composed of pectic substances released from the plant cell walls by the urea in the new extraction procedure.

I tell this story to illustrate how plants can be more difficult to work with than animals. The urea-based procedure had been developed for animal cells, which don't have cell walls or pectin. In retrospect, we think the accelerating effect was probably comparable to that of dextran sulfate - another acidic polysaccharide, somewhat like pectin – which other groups later showed to accelerate hybridization by binding enough water molecules to increase the effective concentration of DNA.

CTAB Procedure. These and other issues led us to develop a new isolation procedure for plant DNA that avoided urea and used a cationic detergent, cetyltrimethylammonium bromide (CTAB) instead of the more commonly used anionic detergent sodium dodecyl sulfate, or SDS. This procedure, which Mike and I published in Nucleic Acids Research in 1977, is by far my most frequently cited paper. The next most frequently cited one is a modified version of the same procedure published in 2006 by George Allen and others in my lab at NCSU. According to ResearchGate, these two papers still get something like 10 or 20 citations per week.

Organelles. Plant molecular biology was still not a heavily populated field during my early days at Carnegie, so it was possible for my group to work on several quite different projects – including all three of the genomes in a plant cell. Jeff Palmer and Neil Woodbury led projects on the chloroplast genome, while David Stern worked on mitochondrial DNA.

David joined my lab while I was on sabbatical at the Plant Breeding Institute in Cambridge, UK. He had been working there with David Lonsdale on maize mitochondria and came to Stanford to do a PhD, leaving Cambridge just before my arrival. I actually worked at his former lab bench. At Stanford, David felt he didn't fit well into the lab where he had originally applied. So – in my absence, and without consulting me – he contacted Winslow about working in my lab. I still remember getting a letter from David (an actual letter; this was well before email) in which he introduced himself and casually stated that he was now working in my lab. And working on mitochondrial DNA, with which I had no experience. I can't imagine this happening anywhere but Carnegie.

When Jeff joined my lab – actually somewhat before David did – he also had a well-developed plan for his PhD research. His plan involved chloroplast DNA, which I'd never worked on. However, he did discuss it with me ahead of time and convinced me that it was an important project and that he could do it. The idea was to do molecular phylogeny with chloroplast DNA. He roamed the Stanford campus collecting plants and analyzed their chloroplast DNA by restriction mapping. That approach turned out to be very useful for phylogenetic analysis in the days before DNA sequencing became commonplace. Jeff was incredibly productive and has continued to be highly productive throughout his long career after leaving my lab. His presence in the lab may have had something to do with attracting Dave Stern, as well as Neil Woodbury, who used then-novel technology to map actual transcription start sites throughout the chloroplast genome.

Jeff's most significant discovery at the time was that pea and its relatives lack a large inverted repeat that was present in most other chloroplast genomes, and that the loss of this repeat was associated with an increase in the frequency of rearrangements elsewhere in the genome. This novel observation led to the first *Cell* paper from my lab.

Light. One can't be around Winslow Briggs for very long without getting interested in the various ways light affects plant development. In my case, this first took the form of characterizing a set of cDNA clones representing phytochrome-responsive mRNAs. Lon Kaufman then led follow-up work establishing that some of these mRNAs were responsive to extremely low amounts of red light – the so-called very low fluence phytochrome response – while others required higher amounts of light. This work resulted in a rather nice *Science* paper and was the basis for a variety of subsequent experiments.

When Mike Dobres joined the lab, he identified one of the cDNA clones as encoding the ferredoxin protein. He, Bob Elliott, and John Watson sequenced it and obtained a genomic clone with a considerable amount of flanking sequence, enabling us to start what turned out to be a long series of experiments on the molecular basis for light effects on this mRNA.

Chromatin. One of the things Mike Murray brought to the lab was an interest in chromatin. Mike introduced me to Steve Spiker, then at Oregon State, and convinced me that (a) chromatin was important and (b) that Steve knew more about plant chromatin than anyone else.

We collaborated on a *PNAS* paper showing that expressed plant genes are located in chromatin with a more "open" structure than the rest of the genome. This had quite recently been shown in animal cells, and ours was the first demonstration in plants.

This result stimulated several other investigations. One was work I did on a sabbatical with Dick Flavell at the Plant Breeding Institute in Cambridge - the same sabbatical in which I worked at Dave Stern's former lab bench. Dick was interested in the phenomenon of nucleolar dominance in hexaploid wheat, wherein certain ribosomal RNA gene clusters are expressed more strongly than others. I was able to show that dominance was associated with a more open chromatin structure. More-or-less simultaneously, Lon Kaufman and John Watson, back home in my lab at Carnegie, showed that rRNA genes transitioned to a more open, less methylated structure when pea bud development was stimulated by light.

North Carolina. It is an oversimplification to say that I tried to get Carnegie to hire Steve Spiker, and we both moved to NC State when that didn't happen. The real story is slightly

more complex. Steve moved a few years before I did. But there is more than a grain of truth in the idea. Certainly, Steve's presence at NCSU was a major factor in my eventual decision.

The first inquiry came from John Mackenzie, who had been a grad student in the Briggs lab when I was there as a postdoc and later was hired at NC State to lead an electron microscopy lab. He called me one day in early 1985 to tell me about a special University Professorship - essentially a professorship endowed by the university - being offered in Plant Molecular Biology. This was part of a legislative initiative in Biotechnology. I'd been testing the job market already at that time, and the job at NC State was by far the best one I'd seen. So of course, I applied.

So - after having thought I would stay forever in Amherst, and then forever in Palo Alto - I moved to North Carolina. Having learned from those other experiences, when I accepted the job in Raleigh I was convinced it was going to be temporary. Wrong again! As I write this, I am a little over six months into my 37th year at NC State.

Just as hiring Mike Murray was the best decision I made at Carnegie, hiring Dolores Sowinski as senior technician and lab manager was the best decision I made at NC State. An MS-level microbiologist, she had skills we needed in a lab in which DNA cloning in bacterial vectors was quickly becoming central to everything. She also brought experience with NC State's bureaucracy, which turned out to be invaluable. Most importantly, she was someone I could really talk with, and who would talk back with ideas of her own.

Dolores coordinated the setup of my first lab in temporary space and did it again a few months later when we moved into "permanent" space. A few years later, she did it a third time, when we moved to the Centennial campus. In addition, she and Bryon Sosinski worked together to set up the Genome Research Lab when Charlie Opperman and I finally persuaded the university to invest in a genomics service facility. Along the way Dolores orchestrated all the everyday operations of the lab and did lots of experiments, too, working on ferredoxin, gene silencing, and other things along the way.

Interdisciplinarity. One of the main reasons I went to NC State was my sense that barriers to interdisciplinary collaboration were lower there than at many other institutions. This proved true, and I was able to spearhead several generations of interdisciplinary training grants from the McKnight Foundation and a "Tri-Agency" consortium involving NSF, USDA, and DOE. Steve Spiker and Arthur Weissinger took turns as co-PIs, and each grant involved about a dozen faculty. We funded something like 15 graduate trainees in each, with the stipulation that each student had to be jointly advised by at least two faculty members. We also required the student projects to be new collaborative initiatives, not previously funded. That led to a rash of new proposals and new grants being funded.

Ferredoxin. Work in the lab continued apace and branched out in new directions. Bob Elliott and Mike Dobres moved with me to NCSU and continued working on the pea ferredoxin gene. Bob led the work that established that light effects were not mediated by the promoter, as they were for most other light-inducible genes, but instead by something in the transcribed region of the gene. Over the years Lynn Dickey played a leading role in extending this work, along with Maria Gallo, Dolores Sowinski, Marie Petracek, and Eric Hansen, among others. We established ferredoxin as one of the first clear examples of post-transcriptional regulation. That was novel even for animals at the time, and we were able to get NIH money to pursue our work on it. Then the animal and yeast worlds surged ahead. We didn't get the NIH renewed, but several NSF and USDA grants kept us going for some years. It was a productive period, with papers in PNAS, EMBO J., Plant Cell, and other notable journals.

Chromatin and MARs. The success of ferredoxin work didn't affect my interest in chromatin. Early during my time at NC State, Steve Spiker went to a Gordon conference and learned about what were then called "Scaffold Attachment Regions," or SARs, which were being shown to have major effects on chromatin structure and gene expression in animal systems. These were supposed to work by attachment to the nuclear scaffold - which later came to be called the nuclear matrix. So, SARs become MARs.

Gerry Hall in Steve's lab and George Allen in my lab did the initial work. Gerry led the identification and cloning of a MAR from a tobacco genomic clone isolated in Mark

Conkling's lab, while George led a project using the "gene gun" to transform tobacco cells with constructs flanked by yeast MARs, finding they enhanced expression.

George and Gerry later showed the tobacco MAR worked even better than the yeast version. And the effects proved heritable. Bekir Ulker led a project showing that MAR effects were apparent in F1 progeny, and Jennifer Levin's work with Arthur Weissinger showed that MARs increased and prolonged transgene-mediated virus resistance at least through the F4 generation.

Multiple lines of evidence led us to believe MAR effects came from reductions in gene silencing. Most of our initial work involved gene gun transformation, which typically produces complex multicopy inserts with a strong tendency toward silencing. However, Luke Mankin showed that MAR effects (albeit smaller ones) were still observed in *Agrobacterium* transformants. Chris Halweg then used flow cytometry to characterize MAR effects in large numbers of individual *Agrobacterium*-transformed cells. Both the frequency and magnitude of transgene expression were increased in transformants made with MAR constructs.

Most of this work was done in partnership with Steve Spiker's lab in Genetics and/or Arthur Weissinger's group in Crop Science. The three of us even worked together with an industrial consortium organized by Mike Murray, who was then at Dow AgroSciences. The whole project was a good example of the interdisciplinary collaboration I had sought by moving to NC State.

DNA Replication. About in the middle of my time at NC State, I was lucky enough to attract Randy Shultz to my lab as a graduate student. That moment was the beginning of the DNA replication phase of my career.

It didn't happen instantly. Like most graduate students, Randy tried out a project or two that didn't catch fire. But then he took a course with Linda Hanley-Bowdoin and wrote a class proposal about mapping DNA replication origins in *Arabidopsis*, following the example of a pioneering paper in yeast. Linda liked his proposal enough to suggest that Randy and I collaborate with her on an actual proposal to NSF. As they say, the rest is history.

At that time, in the early 2000s, no one had studied replication programs in plants. So we - Randy, Linda, and I - worked up a proposal for the NSF Plant Genome Research Program. We were lucky that program existed because the embryonic state of replication work in plants might have doomed us in a broadly focused panel like Eukaryotic Genetics. I owe many thanks and offer my sincere admiration to Mary Clutter, Machi Dilworth, and others who created the PGRP. Its impact on plant biology has been immeasurable.

That first proposal, ultimately submitted in collaboration with Rob Martienssen, was almost totally lacking in preliminary data. But it got us a small "sugar" grant (SGER, for Small Grants for Exploratory Research), with the idea that we should use that money to get the needed preliminary data. Thanks to Randy and Tae-Jin Lee, who joined the lab at that time, and to Pablo Rabinowicz in Rob's lab, we were able to generate enough data for our first real grant on this project.

We are now working on our third major PGRP grant (not counting the SGER). Co-PIs have included George Allen and Bryon Sosinski at NCSU, Rob Martienssen at Cold Spring Harbor, Matt Vaughn (first at CSHL, then at Texas Advanced Computing Center), Dorrie Main at Washington State, Jawon Song at TACC, Hank Bass at Florida State, and Lorenzo Concia at TACC.

We first worked on Arabidopsis, using cells growing in suspension culture to facilitate labeling, and published the first characterization of a plant DNA replication program. Tae-Jin and Pete Pascuzzi were co-lead authors, along with a cast of thousands.

Lorenzo Concia then led a further, more detailed characterization, and Emily (Markham) Wheeler led work characterizing regions in which replication initiates in very early S-phase.

Dolores retired a few years after the replication project started, and I was very lucky to hire Emily Wear, and, later, Leigh Mickelson-Young for the last phase of my time at NCSU. They have both been wonderfully creative, hard-working, and skillful. I'm extremely grateful because there is no way I could have kept things going without their help.

After Arabidopsis, we turned our attention to maize. There we developed a root tip system to study DNA replication in the context of an intact plant organ. In collaboration with Hank Bass at Florida State, we showed that the spatio-temporal patterns of replication in maize nuclei

were different from those in typical animal cells. Emily Wear then led projects describing the molecular pattern of replication in maize and comparing replication patterns in mitotic and endocycling cells. Emily, Leigh Mickelson-Young, and Emily Wheeler continue to lead exciting projects at NCSU. We continue to collaborate with Hank Bass at FSU and Lorenzo Concia, who is now at TACC. (Both Hank and Lorenzo are now co-PIs.) Linda Hanley-Bowdoin has taken over as lead PI - and as I approach my 80th year I'm very grateful that she has. I'm equally grateful that I haven't had to give up participating in the science!

Nearing the End. As I write this, it is a little more than 60 years since I started college with the idea of becoming a plant ecologist. It has been a long and winding road, and I never did become an ecologist. However, I consider myself fortunate to have been able to pursue many different projects in molecular biology.

Crucially, I have also been supremely fortunate to have had many outstanding students and colleagues with whom to pursue those projects. I think a key part of academic success is allowing one's students, postdocs, and other colleagues to bring new perspectives and approaches. I am deeply grateful to all those who challenged me with intriguing questions and creative ideas. And I'm still excited by what we might find around the next corner!