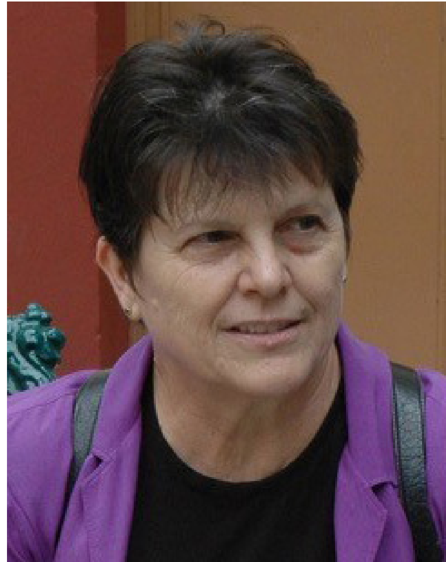
ASPB Pioneer Member

Carolyn Napoli

My journey through higher education was somewhat atypical. After high school, I went to business school, married, had a child, and divorced. During my high school years, I was more interested in art than biology. While working as a secretary, it became apparent a college degree would be essential to gain control of my future, so I enrolled in the local Junior College, my only option at the time. In my first class, the reading assignment was "The Microbe Hunters" by Paul de Kruif. I was fascinated and enthralled by the hunt, and it awakened in me a problem solving desire that would persist throughout my scientific career. I knew immediately what I wanted to do: I would earn a PhD in microbiology. This was a lofty goal for a single mother with no money other than that from a part-time job. How could a student with no biology background and little money obtain the highest degree awarded by a university? I knew it would require great effort, persistence, and resolve, but I grabbed the bull by the horns with enthusiasm.

Once I obtained my AA degree, I transferred to the University of Florida (UF). Fortunately, I was awarded a scholarship to pursue a BS in Microbiology, which I obtained with Honors in 1972. DeLill Nasser, at that time a faculty member in Microbiology, was an important advocate for my enrollment as a graduate student in the department. In fact, she was instrumental



in securing a part time position in David Hubbell's lab in the Soil Science Department while I was an undergraduate. I continued in Dave's lab for my PhD research and investigated the process of infection of *Trifolium* (clover) roots by the soil bacterium, *Rhizobium trifolii*. Using electron microscopy, I was able to demonstrate, for the first time, the development of infection threads within root hairs. Another project showed the novel observation that root exudates can promote a more rapid formation of infection threads in root hairs, implying the existence of a signal that stimulates the Rhizobium-root hair interaction.

As root hairs are the specific site of rhizobial infection, I felt a comparative analysis of carbohydrate profiles from root hairs and epidermal cells could reveal important differences in cell wall composition and lead to insight as to why root hairs were the infection target. I obtained an NSF postdoctoral fellowship to continue Rhizobium research in the laboratory of Peter Albersheim at the University of Colorado (CU) in Boulder to study this subject. Preliminary research indeed revealed some carbohydrate differences. Further research would center on carbohydrate chemistry profiling, as well as identifying the carbohydrates involved. Of course this would require training in a new field, carbohydrate biochemistry. I realized I was at an important transition point that would determine my future career path, so I began to rethink my interests. I was becoming aware of the advances and power of molecular genetics, and I concluded that if I were going to transition into a new field, the field should be molecular genetics. Of course CU was a hotbed of cutting edge molecular genetics research, so I simply needed to move over to the Department of Molecular, Cellular and Developmental Biology. I joined the laboratory of Larry Gold, and brought along my new NIH fellowship.

Larry had an incredible group of students, postdocs and staff. The lab meetings were stimulating and educational. With Larry's guidance, I chose a project involving reinitiation of RNA synthesis at the rIIB cistron of bacteriophage T4, based on the work of Stahl, Crick and Brenner in "The Genetic Nature of the Genetic Code for Proteins". Their elegant experiments used frameshift mutations to deduce the triplet nucleotide coding for protein synthesis. In the course their work,

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Stahl *et al*. observed tiny mutant phage plaques (as opposed to the normal robust ones), which they named "minutes". They hypothesized that translational reinitiation events, following premature terminations in the rIIB coding region, resulted in the mutant plaques. My research tested this hypothesis by way of DNA sequencing and polyacrylamide gel (PAGE) analyses of truncated proteins and confirmed translational reinitiation occurred at specific sites. DNA sequencing by the Maxim-Gilbert method was still quite new then, but being that I was not pleased with the amount of time required to develop the autoradiograms, I set up the even newer method of Sanger sequencing, which gave results within 24 hours rather than days. Word of this spread around the department, and Sanger sequencing was soon adopted throughout the department.

While the research and Larry's group were great, I knew I needed to move on and secure a better paying job to support my daughter. And so, I explored possibilities in the new and burgeoning biotech industry, ultimately accepting a position at The International Plant Research Institute (IPRI) in San Carlos, California. I cannot say much about my time there. Unfortunately, research-wise the company was chaotic and floundered and so did I. However, one thing that went well was the side work I did on bacterial avirulence for a fellow staff member, Brian Staskawicz. When Brian accepted a faculty position at the University of California, Berkeley, he offered me a position in his new lab, working on plant avirulence genes. During this time, I met and married Rich Jorgensen, who was working in Oakland at AGS (Advanced Genetic Sciences, Inc.), which was acquired later by DNAP (DNA Plant Technology Corporation). Through Rich, I was introduced to the great possibilities arising at AGS, given the new methods for Agrobcaterium-based transformation of plants and the first rate scientists at the company, especially Hugo Dooner, Jonathan Jones and Caroline Dean. I soon joined Rich to establish a program to engineer floriculture crops. Our main focus was on engineering flower petal pigmentation in high-value crops such as carnation, chrysanthemum and rose. For proof of principle, we chose petunia as a model system, because a transformation method existed for petunia (but not yet any high-value floriculture crops). Also, the anthocyanin pathway responsible for flower color was well characterized in petunia. It had already been demonstrated by Joseph Mol's lab in Amsterdam that an antisense Chalcone Synthase (CHS) sequence driven by the constitutive 35S promoter could reduce pigmentation in petunia flowers.

Our goal was to over-express the gene encoding the CHS enzyme and hopefully increase flower pigmentation by use of a 35S-driven over-expression vector for high level translation designed by Jonathan Jones. The complete CHS coding sequence was fused in an optimal translation initiation context, since our objective was over-expression of a functional protein and not merely the transcription of RNA. Christine Snyder introduced this construct into petunia leaf explants and regenerated whole plants. As an aside, we decided to make and introduce an antisense construct, simply to repeat the Mol lab antisense experiment. Although our hypothesis was that the sense CHS over-expression construct would intensify flower color, we were astounded to see that the flowers were completely white in some transformants and produced developmentally controlled white-on-purple patterns in others. The antisense CHS construct created a completely different effect, with irregular, lighter petal pigmentation; no white flowers were observed. The over-expression construct was even more effective at blocking pigmentation than the antisense construct! To determine that a mutation had not accidentally been introduced into the CHS coding sequence during vector construction, I sequenced the DNA insert of the final construct to show it was not mutated. My colleague at AGS, Carol Katayama, suggested RNase protection would be a precise experiment to look at RNA abundance. To this day, I remember the sense of exhilaration upon developing the autoradiogram and seeing the result in the dark

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room. There was very little endogenous and very little transgene CHS RNA in the white-flowered plants, whereas in other transformants producing normal, purple flowers, the endogenous CHS RNA levels were high (wild-type) and the CHS RNA level from the introduced sense construct was also high. In other words, in the purple flowers the two genes' RNAs were co-expressed, while in white flowers both genes' RNAs were co-suppressed! As I stared at the results, I felt a profound shift in gene expression control had taken place causing this result. We submitted a poster abstract for the 1990 Plant Molecular Biology Keystone Conference. A select number of poster presenters were asked to give a short talk, and as first author, I was invited to make the presentation. However, I felt Rich could better convey the significance of the discovery, given his much longer experience in plant molecular biology, genetics and development. He gave an excellent presentation and ultimately would receive the bulk of the recognition for the discovery. That was fine with me, as I had only just recently become a plant molecular geneticist and was still a neophyte in plant development.

Rather than remain an industry genetic engineer, I had long yearned to be a profesor, so I could teach, train, and guide students, as well as carry out purely academic research. Given my path to that point, I felt I had unique perspectives to contribute to the next generation of students. Although I knew DNAP would not allow me to take the co-suppression research with me, I was able to use co-suppression for my presentation as a job candidate. Subsequently, I accepted a faculty position in the Department of Environmental Horticulture at the University of California, Davis (UCD). In my job interview, I proposed to develop a new research program in plant developmental genetics. My art doodling as a child had often consisted of trees with varying branching patterns, and so during my research at DNAP I couldn't help taking an interest in petunia's distinctive pattern of lateral branching during vegetative development. It was obvious to me that I should use a genetic approach as the means to identify genes involved in branching. As an aside, I eventually convinced Rich to see the light and move back to academic research. After a year, DNAP acquiesced and allowed him to take a number of transgenic petunia lines to UCD. He was offered an unfunded position in the Vegetable Crops Department, but he was not allowed to grow petunias in their greenhouses. So he transferred to Environmental Horticulture, where growing petunias was not frowned upon. This also made it easy for us to continue collaborating on co-suppression.

Upon my arrival at UCD, I undertook ethyl methanesulfonate (EMS) mutagenesis of both petunia and Arabidopsis seeds. I quickly found three petunia mutants with bushy phenotypes among the first 400 segregating M_1 families, but none were observed in thousands of Aradidopis families. Further screening of a total of 1,283 independent segregating petunia M_2 families produced a total of nine bushy plants, which I termed *decreased apical dominance (dad*) mutants. Genetic analyses confirmed the existence of three independent recessive loci responsible for increased basal lateral branching. Six alleles of the *dad2* gene were unstable and spontaneously reverted to wildtype, suggesting a repeatable transposition insertion event rather than point mutations. The dad1 phenotype was found twice and the dad3 phenotype once; these mutants had stable branching patterns.

Christine Beveridge at the University of Queensland in Brisbane (Australia) had used grafting to demonstrate that the bushy *Pisum sativum rms-2* mutant phenotype was restored to a near wild-type branching pattern, indicating a mobile signal was involved in control of lateral branching. I used this information and performed extensive graft analyses to further characterize the DAD mutants. I devised a technique wherein I performed grafting with axenic seedlings on petri dishes containing water agar as the substrate. After the graft unions healed, the seedlings were transferred to soil. All combinations of *dad-1*, *dad-2*, and *dad-3* were constructed with wild-type rootstock (scion). Both *dad1*

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and *dad-3* mutant scions reverted to normal lateral branching when grafted onto wild-type root stock. The *dad2* phenotype was not reversed and grafting wild-type scions onto mutant rootstock did not alter the phenotype. Reciprocal grafting of *dad-1* and *dad-3* failed to produce a wild-type branching phenotype. The results suggested the genes could be involved in the same pathway. I took the graft approach one step further and used "interstock" grafting. With interstock grafting, a small piece of wild-type stem was inserted in between the severed hypocotyl of a *dad-1* seedling. The result showed the small wild-type stem fragment reversed the bushy phenotype to wild-type. In instances where roots developed above the interstock, the mutant bushy phenotype reappeared. When the wild-type interstock was introduced above the first node of the *dad-1* seedling, mutant branching was observed from the two distal nodes; but the mutant stem above reverted to the wild-type pattern as long as roots were removed above the interstock. The later experiment showed a signal from the wild-type interstock was controlling lateral branching, and furthermore the signal moved acropetally not basipetally.

Many other types of mutants were found among the segregating M2 families. I focused on a few select non-bushy mutants. One named *white anther* (*wha-1*) had slightly reduced purple flower color and white pigmented anthers, as opposed to yellow wild-type anthers. I established a collaboration with Loverine Taylor at Washington State University, and we demonstrated the lesion was in CHSA, one of the two chalcone synthase genes expressed in petals and anthers. This mutant provided a valuable phenotype for her male sterility research. Another mutant named *sympodial-1* (*sym-1*) failed to establish wild-type sympodial branching during the flowering phase of growth. My graduate student, Jon Ruehle, worked on this mutant for his PhD thesis. Another mutant derived from an outcrossing study displayed a mutable flower color phenotype similar to the action of a transposon. My postdoctoral fellow, Kimberley Snowden, confirmed the phenotype was caused by a transposon insertion within the 3′,5′-hydroxylase gene. We hoped the transposable element could have utility for gene tagging in petunia; Kim determined the element was a member of the maize *Spm* transposon family. But before we could do further work on the utility of this transposon, I had to leave Davis.

Using further EMS mutagenesis, I used the dad-1 mutant plant to see if a revertant to wild-type branching existed. However, growing hundreds of highly branched *dad1* plants (F_2 population) could be problematic. I used 100 *dad-1* seeds for a trial mutagenesis. In this small F_1 population, there was one revertant to a nearly wild-type phenotype, and of course it was a dominant mutation. What I began to realize was that EMS had likely not produced just a series missense or nonsense mutations, but the chemical must have also unleashed transposons to induce mutations in some instances.

Upon arriving at UCD, I was surprised to find I was expected to support other faculty members' molecular genetic research projects and train their students in molecular biology. In fact, there were students residing in what was supposed to be my lab, none of whom worked in my research program. The presumptiousness of it shocked me. Apparently, I was expected to simply be the departmental molecular biologist and collaborate with other faculty rather than develop my own research program! Perhaps because I refused to submit to their wishes, the department did not support me for tenure (split decision), even though my outside letters of recommendation were extremely positive, one even calling me a pioneer. By contrast, the important campus-wide peer review committee supported my promotion, as did the Dean of the College. Nevertheless, I was denied tenure by the Vice Provost. Bill Lucas was disturbed by this and organized a letter writing campaign by my campus peers in support of my tenure. It was to no avail, as someone higher up wanted me gone. In my termination letter, the Chancellor stated he was not impressed with my research. This was despite the support of my

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peers (both outside letters and support from many colleagues on campus), as well as my success in securing funding by NSF, an initial grant and a renewal.

Thanks to strong support from Brian Larkins, Vicki Chandler, and the department head, Rob Leonard, Rich and I were able to transfer our research grants to the Department of Plant Sciences at the University of Arizona (UA), where we were given non-tenure track research faculty positions. The move to Tucson was very positive, and we were enthusiastically welcomed and given brand new labs. My postdoc, Kim Snowden, came with me and we were off to a great, fresh start. However, something began to go wrong with my health. I learned my adrenal glands had become suppressed by constant use of prednisone to treat the severe asthma that developed from California's Central Valley air pollution, which permeated Davis. Tucson, by contrast, had good air quality; my asthma was not a problem and prednisone was no longer necessary. However, my adrenals had become suppressed by the use of prednisone all those years, and I became exhausted if not taking prednisone to stimulate enough adrenaline to be able to function.

The tenure debacle and the move to Tucson were significant dissruptions to my research program, and it was apparent that Kim and I could not produce sufficient new results to be competive for an NSF grant renewal. Kim returned to New Zealand, and after she obtained a position at the HortResearch Institute in Auckland, I encouraged her to take over the petunia lateral branching project. She agreed, and not only did she continue the work, she went on to be very successful characterizing and cloning all three *dad* genes and became a leader in the field.

After two years, my adrenal function was finally back to normal; thus, it did not end my academic research career. The start of my next incarnation in science commenced when I designed and supervised plasmid and vector constructions for a multi-university NSF Plant Genome Research grant designed by Rich Jorgensen and Vicki Chandler. I was provided a lab and two technicians, and my group focused on preparing plasmid constructs containing inverted repeats (IR) of select chromatin coding sequences for RNA silencing in Arabidopsis and maize. In collaboration with Judith Bender, Eric Richards, and Craig Pikaard, the team made hundreds of IR constructs, introduced them into Arabidopsis and provided transgenic seeds to the Arabidopsis community and gene constructs to the maize community. Additionally, I became active in ChromDB (The Plant Chromatin Database), which was a key part of the project directed at identifying and organizing sequences of Arabidopsis and maize chromatin related proteins and genes and to make them available to the community. This also involved building molecular phylogenetic trees of the larger gene families, such as histone acetyltransferases and deacetylases.

For the renewal of the Plant Genome Grant, Rich and I decided ChromDB should stand on its own, separate from the genetics part of the project. As PI, I obtained an NSF Plant Genome Research grant to continue developing the ChromDB database/website and expand coverage to all sequenced plant genomes, as well as many fungal and animal genomes. ChromDB was highly successful and an internationally used website and database that provided easy access to a large number of chromatin genes that were organized into families and subfamilies, showing protein domains and known cDNA sequences. As well as being a service to the community, Rich and I used ChromDB to further analyze the evolutionary diversification of chromatin genes in plants, animals, and fungi. A number of other databases adopted and utilized ChromDB classifications and information.

I am especially proud of the high school educational outreach funded by the grant, which provided high school science teachers with a summer internship to participate in bioinformatics and molecular genetics experiments. Additionally, with the aid of the teachers, we developed a protocol for experiments to be conducted in high school classrooms, whereby students would amplify sequences from the RNA of select chromatin

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genes to provide proof (or not) for the splicing model predicted by the sequencing algorithm. We developed a manual to accompany the in-classroom experiment. The classroom experiment was a success. The coding sequences were deposited at GenBank (under the name of the classroom teacher to avoid privacy issues with minors), and the manual was delivered to the NSF Program Manager, Diane Okamuro.

I had a Masters' student, Tara Paulsen, who developed an algorithm using the highly conserved pfam protein motifs, as well as the spacing between motifs, to proof the splicing models. Our evolutionary work added in this process, as we determined if the protein families were highly conserved. With the aid of a mathematician, we were able to successfully design an algorithm that confirmed or discounted a model. Karla Gendler was the bioinformaticist on the ChromDB project, and she produced stellar work on the database and provided invaluable advice on the algorithm. ChromDB would not have existed as a community resource and research tool without her intelligence and programming and informatics skills.

I applied for a renewal of the ChromDB grant with Shelly McMahon as co-PI. Shelly's research interests centered on large scale phylogenetic analyses. The goals of the grant were to continue providing new genome sequences to the database, continue creating tools for community usage and the evolutionary work, but especially continue development of the algorithm to "proof" splicing models. Unfortunately, the renewal was denied. The panel summary contained the line, 'they curate the database with love'. Did love, not science, establish an internally recognized database, a richness of evolutionary data, and the means to correct splicing models? All my creative work, as well as that of my group, was reduced to an emotion. I could no longer endure science. To this day, I continue to be amazed that a program manager at NSF would allow such an unprofessional comment to be included in a panel summary. I retired in 2010 when the ChromDB genome grant funds ran out.

My biographical profile reflects a disjointed and wandering path, albeit a very rewarding one most of the time. By necessity, I successfully reinvented myself a number of times throughout my career. I always searched for excellence and creativity in science, and I believe I found it, especially with cosuppression, my lateral branching work and the scope of the ChromDB database as a research tool. While attending a meeting of Plant Genome Research PI's at NSF, I encountered DeLill Nassar again for the first time since I met her at UF at the beginning of my scientific career. I was thrilled to hear her say how proud she was of me and that I was one of the success stories of the Microbiology Department at UF.

While gender equality has progressed since the early days of women pursing science careers, it was still there when I started my education. They let us in the door slowly, but continued such practices as a male committee members asking female students out on a date and a professor draping himself over a female student to show her how to use the dials on an electron microscope. In the 1970's, many women were on the cusp of pursuing advanced university degrees in science. Some of us prevailed, but sadly some did not. Why? Was it gender discrimination? Was it a lack of mentoring? Was it a lack of determination? I often encountered the first two, but I stubbornly pursued the goal that I was unwilling to let go, even when I was told I had to be ten times more qualified than male applicants to be admitted to the Department of Medical Microbiology at UF. Women who entered academic science when I did definitely had more and higher hurdles than did their male colleagues.

In closing, I want to acknowledge the most important person who made it possible for me to obtain a PhD degree and pursue my career, my daughter Anne-Marie Napoli. She was a wonderful child, so independent, loving, and understanding. If she were not, I could not have focused nearly enough energy and time on school. She is the best part of my life and my best friend. Bless you Anne-Marie and thank you.