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Darcy Kelley's research has elucidated the broad range of biological mechanisms—from hormones to genomics—that shape innate vocal communication. She developed Xenopus as a biological model for developmental, cellular, and molecular explorations of sexually differentiated behaviors. She and her laboratory members identified mechanisms through which gonadotropins, androgens, and estrogens shape auditory processing and vocal pattern generation. Together they developed powerful ex vivo approaches to identify evolutionarily conserved components of vocal neural circuits from forebrain to hindbrain. Kelley and former trainees—now tenured faculty members in the United States and abroad—collaborate in developing new approaches for identifying how neural circuits for vertebrate social communication evolve. Her current research foci include genetic mechanisms that support the divergence of vocal communication during speciation and the biology of neurodevelopmental disorders of social communication. She has served as trustee of the Marine Biological Laboratory, the Grass Foundation, and the Association of American Colleges and Universities.

Darcy Brisbane Kelley

Family

I grew up in New York City. My mother's family (Brisbane) was from upstate New York and my father's (Kelley) was from New England. My mother Elinor was the youngest of six, and her father Arthur died on Christmas day when she was 11. My grandmother, Phoebe Cary, was ill throughout my childhood; I did not know my maternal grandparents. I was, however, very close to my paternal grandmother, Harriet, whom I named "Gaga." Gaga grew up in North Attleboro, Massachusetts, but was a frequent visitor to her aunts in Brooklyn and loved New York City. After she had broken off several engagements, my great-grandmother Maud ("Mother") Tweedy ordered Gaga to accept her most recent offer: from my grandfather, Solon Kelley II, a New York City stockbroker. When he insisted on moving the family to Connecticut, Gaga left him and supported herself by working at Macy's. Her sons (my father and his younger brother Ned) had to live with Mother Tweedy until they were dramatically rescued in the midst of a snowstorm by Gaga and her new husband, Freddy Wildman. Mother Tweedy was so terrifying that whenever we had to visit, my mother and I would hide inside a car in her garage.

My first year in graduate school, grandfather Solon came to visit. Over a luncheon (I figured it was now or never), I asked him what had happened with Gaga. Grandpa said that he had followed Harriet to Paris (she had to go to France to get a divorce). When he caught up with her on a bridge over the Seine, Harriet took off her wedding ring and threw it into the river. He only saw her again once, at a party. Grandpa was ascending the up staircase and Gaga descending the down; she winked at him and went on. Before I headed back to Rockefeller, Grandpa asked me whether I was going to be a "bluestocking": an old-fashioned term for a scholarly woman who never marries. I had to agree with Gaga; Freddy was much more fun.

My father (Solon III) grew up with Gaga and Freddy in New York City. He was educated at the University of Virginia before leaving to join the Army Air Corps (later the Air Force) at the beginning of World War II. He was a decorated fighter pilot, served in the China-India-Burma and European theaters and was released with the rank of major. My parents met after the war, married in 1947, and started their family: me (the oldest), my sister Harriet (the artist Mandy Staffa), and my brother Soso (Solon IV, and the last). I do not know why they were all named after a lawgiver in ancient Greece. My father never discussed his war service but continued to fly, piloting us to Martha's Vineyard to vacation with my cousins Allaire and Cary; my mother was very close to her sister Alice. My father died of

bladder cancer at 51 during my first year at Rockefeller. He was the last living member of his squadron; they all died of the same disease. Air Force and Navy pilots continue to die of bladder cancer to this day.

My mother Elinor also grew up in New York City; she graduated from Bennington College in Vermont. Bennington attracted outstanding faculty during the war. W. H. Auden delivered a series of lectures that contributed to my mother's desire to be a writer. Her thesis on "The Brothers K" (Dostoyevsky) was supervised by Peter Drucker, professor of politics and philosophy at that time (and later a management guru). Solon joined his stepfather, his brother Ned, and his half brother Freddy Jr. to form Wildman and Sons, a wine importing firm. Freddy Sr. served as both a lieutenant with the Second Division in France in World War I and then as an Air Force Colonel in World War II. According to family lore, after World War II ended, "Big" Freddy somehow secured a car and drove—from one notable French vineyard to another—lining up the wines that Wildman and Sons would later import. Big Freddy was a man of considerable charm: a "grand seigneur." My grandmother loved to travel, and their partnership was strong.

The Wildman and Sons trips through Europe were a frequent part of our childhood and usually included my mother, who had spent a year in Versailles as a child and so was fluent in French. After one long separation, we children pretended not to recognize our parents. This move did not have the desired effect. We did, however, get to tag along once for a summer vacation in France at Pyla sur la Mer where I learned to play couteau (a child's version of mumbley peg) on the beach. Stony Beach in Woods Hole, where I later spent summers with my own family when at the Marine Biological Laboratory (MBL), always brings back memories of that beach in France. If you have read the opening of Lewis Thomas's *Lives of a Cell*, you might see the resemblance, which is one reason why I have always been so happy at the MBL.

Throughout my childhood, we lived off of East End Avenue. Before the East River Drive was built, our apartment building fronted directly on the river, and tenants could dock their boats. The bottom part of the building was later converted to fallout shelters that my friends and I would explore on rainy weekends; they were stocked with supplies during the Cuban missile crisis while John F. Kennedy was president. Rockefeller also had subterranean domains with a series of tunnels. While exploring them with my fellow graduate students, we once found one that led to a cowshed, erected for a cell biologist studying the pancreas, if memory serves.

Education

When the time came for first grade, my mother sent me to the Chapin School because the headmistress, Miss Stevenson, had said she would watch

out for me as I crossed East End Avenue. I was a moody child, and Chapin was a good choice. My education led me to believe, despite growing up in the 1950s, that women could be powerful and erudite leaders. Chapin was run entirely by women; the only men were talented musicians (Robert Schrade and Charles Walker). Miss Stevenson succeeded Miss Chapin and in turn transferred power to Mrs. Mildred Berendsen, a most formidable leader, whom we called Milly Bear. Mrs. Berendsen's subject was history, and seniors had the privilege of taking it in her office. My other teachers, equally demanding, were kind.

I developed a passion for science beginning at age 11. My inspiration was a renowned, French–American child psychiatrist: Dr. J. Louise Despert. I met her because she had written a book for parents, *Children of Divorce*. One evening in 1959, our parents invited us to sit down and then announced that they were getting a divorce. My brother and I reacted to this unexpected news by developing hysterical paralysis and my sister looped the cord of the venetian blinds in our shared bedroom around her neck. Our mother had to bribe us with treats to go talk to Dr. Despert every week, but eventually we felt better; she was a skilled clinician. As I recovered, we'd discuss the science books I took out each week from the library on my walk to her office. Dr. Despert remained a lifelong friend of our family, inscribing another book—*The Inner Lives of Children*—to my son Alex when he was born in 1975. Until redirected as a teenager, I wanted also to be a doctor and a child psychiatrist.

Despert was also a distinguished and prolific academic psychiatrist, widely recognized as an expert in childhood schizophrenia. In the Ackerly oral history project on "Pioneers of the Child Guidance Movement" (William Gardner Collection, Kornhauser Health Sciences Library Historical Collections, University of Louisville), Despert recounts leaving her medical studies in Paris to become a nurse during World War I. She moved to New York in 1920 and then completed her studies at Barnard and NYU Medical School, paid for—as she told my mother—by a grateful patient from her days as a nurse. In her 1971 paper reflecting on infantile autism, Despert describes the link between aversion to cuddling in infancy and complete lack of sexual drive in adulthood and asks, "Is there not something lacking, some hormone perhaps, an ingredient which interferes with the early affective development by its very absence?"

My newfound interest in science was tolerated graciously, although my English teacher—Judith Phelps—said that I gesticulated too much to ever be a doctor. I learned to think and to write, however imperfectly, over the course of my 12 years at Chapin and to deeply respect educators. Mathematics was not my strong suit except for geometry, but I did advance in calculus thanks to a brilliant teacher, Ruth Hutter. I ran into Mme Meller, my French teacher, on the bus some years ago, and she complained about the ascendance of computer "languages." French was very handy though

during our later fieldwork in Gabon. I keep Chapin's motto—*Fortiter et Recte*—in mind; it helps during tricky situations at Columbia.

"Here's the thing," as we say today. In the 1950s, women worked when they had to support themselves and their families. Only some jobs were available to them, but these included teaching, not just as a job but as a profession, and a powerful one. As Columbia students, Bridges and Sturtevant decided to join Thomas Hunt Morgan's lab after taking introductory biology from him—the only time Morgan ever taught it—and not after hearing a research talk. They liked the cut of his jib.

I began my own teaching by tutoring college classmates in Biology I in the dinner line at Grinnell College, gave a lecture on bats in Biology II at Barnard, and was teaching assistant in a neuroscience course at the New School while a first-year graduate student at Rockefeller. Today, many of my Columbia Medical School colleagues believe that teaching interferes with research: why teach when you could be in the lab? However, learning about something new to teach it can pave the way for new research directions. When I moved to Columbia in 1983, I began teaching developmental and systems neuroscience to undergraduates. A few years ago, I added viral vectors to my lecture on tracing neural circuits, and so I had to learn virology. Then I joined forces with other amphibian research labs to develop viral vectors for frogs and newts. Next, came Covid. Ironically the question of how this virus jumped from bats to humans—changing its host specificity—is basically the same problem as figuring out how viral vectors that transduce mouse neurons can be biologically reengineered to figure out fascinating questions in newt and frog brains: regeneration of adult brains, evolution of cortex, and genetic control of circuits for innate behaviors.

The 1960s

I was a teenager in the 1960s: Selma and Martin Luther King Jr. marches and protests. My father was conservative; he hated my brother's long hair, and we fought bitterly about the Vietnam War. My mother, though, was a "total liberal." The father of my classmate, Christina Berlin, was a newspaperman and took his daughter with him to meet the Beatles. Everyone in my class was sick with envy. With my close friend and classmate, the painter Adele Alsop, and some musical buddies, we'd roam across the NYC boroughs to play folk music. Our banjo player, Joseph Pennock, introduced us to Flatt and Scruggs backstage at Carnegie Hall. I tutored kids, supported the Student Nonviolent Coordinating Committee, and watched the assassination of JFK on black-and-white television.

In 1957, the Soviet Union launched *Sputnik*, creating consternation in the United States; clearly, we had fallen behind the U.S.S.R. The National Science Foundation (NSF, only just founded in 1948) was moved to sponsor summer science programs for high school students. By then, I had become

interested in biology, and endocrinology specifically, through reading *Nobel Laureates in Physiology and Medicine*, especially the part on Banting and Best's discovery of insulin. In 1965, as a high school junior, I applied to two NSF-supported summer science institutes: one at Mt. Holyoke on chemistry and another at Grinnell on the biological basis of behavior. I didn't know that behavior *had* a biological basis. Luckily, as chemistry is not my thing, I chose Grinnell and, having passed the required psychological test, traveled to the cornfields of Iowa.

Neil Kent's NSF Behavior Science Institute at Grinnell College aimed to prepare high school students with scientific potential to major in psychology as undergraduates. The test (of religious beliefs) weeded out applicants that might have a problem with an assigned text: *Walden II*, B. F. Skinner's Utopian novel. D. W. Tyler taught us to operantly condition "rats" in simulated experiments and use statistics to analyze the results. The Rutgers ethologist, Danny Lehrman, traveled to Iowa to talk to us. He did a great impression of bow-cooing in ring doves. The experimental psychologists Ogden Lindsley and Nate Azrin described using operant conditioning to shape behaviors of autistic children. Arnie Kriegstein, now a stem cell neuroscientist and former Columbia colleague, was in my Grinnell cohort, as was the psychologist, Michael Domjan. And I did end up majoring in experimental psychology because neuroscience and behavior majors did not yet exist. The upshot was that I decided to matriculate at Grinnell because I could do research as an undergraduate. I withdrew my application to Radcliffe, and even tried to skip my last semester at Chapin to start college early. Mrs. Berendsen talked me out of it; among other arguments, I was school president. My yearbook motto was "Dux femina facti" (from Vergil's Aeneid, chapter 1; roughly "The leader of the exploit a woman").

It is hard to imagine now how different the 1950s and 1960s were, especially for women. You can get some flavor for the times by watching "The Marvelous Mrs. Meisel," although it wasn't as much fun in real life. In 1966 in Iowa, women students had "parietals" and had to be back in the dorms by 10 p.m. on weekdays and 11 p.m. on weekends. We had bed checks; the men were still outside serenading the women who were leaning out of the dorm windows. Girls got pregnant and dropped out of college to marry, so parietals and bed checks clearly were not doing the job. I gave evening talks on birth control in the lounges. Coeducation had other curious features that I had not encountered at Chapin. Girls, even the most brilliant ones, almost never spoke up in class. This experience, and a book, *Sex and Internal Secretions*, awoke a life-long interest in the biology of sex differences.

Barnard College

My father's illness worsened, and in 1968, I returned to New York, transferring to Barnard College as a second semester sophomore. Columbia did

not admit women until after I joined their faculty in 1983. I was a psychology major, in part because my NSF summer institute allowed me to skip introductory psychology. However, while at Grinnell, I had also read Don Griffin's book (*Listening in the Dark*) on bat echolocation and was entranced by the ability to paint a picture of the world in sound. I devoured everything I could read about bats. I wanted to find out how echolocation developed in baby bats, using the classic paradigm for discerning innate behaviors: social isolation in infancy. Barnard's introductory biology course (the one I gave the bat lecture in) was taught by David Ehrenfeld (now a noted conservationist at Rutgers). Ehrenfeld, who studied sea turtle navigation, called Don Griffin (see Volume 2), who had been recruited to Rockefeller from Harvard, and arranged for us to meet.

When I walked into Griffin's lab in Smith Hall, in 1969, a group was clustered around a tape recorder. Roger Payne had just returned from recording whale songs; beautiful, mysterious, very low-frequency sounds. Griffin suggested that I contact Marta Nottebohm, the wife of my future postdoctoral adviser, Fernando (see Volume 8), as she had hand-reared baby bats during one of their field expeditions. Richard Penney, stationed at the NY Zoological Society, knew of a colony of *Eptesicus fuscus* (big brown bats) in Purdy Station, northwest of New York City. We met at the Institute for Research on Animal Behavior at the Bronx Zoo, Penny drove me and Marta to the barn to get the bats, and we returned with three. Marta gave me tips on feeding them (tiny baby bottle) and also on getting the baby bats back to New York City safely. This trip later produced the infamous "bats in the bra in the subway" story that I gave yearly when I lectured to first-year Columbia students taking "Frontiers of Science." From sound spectrograms made in Griffin's lab, I could see baby bat ultrasonic calls shortening daily, supporting the idea that the capacity for producing the short chirps of echolocation sounds was innate.

Don Griffin also introduced me to Jim Simmons, a psychology grad student of Glen Wever's at Princeton. I graduated from Barnard a semester early and traveled to Europe with Simmon's group to collect the horseshoe bat, *Rhinolophus ferrumequinum*, at a field station in the mountains between France and Spain. Our guide, the subterranean insect expert, Michel Bouillon, took us to private caves where we wiggled though tunnels to see prehistoric cave drawings illuminated by smelly acetylene headlamps. We returned to Paris by train with the bats and stayed with our host, Rene Guy Busnel; and I went to my first scientific meeting. *Rhinolophus* has a two-part echolocation call: constant frequency followed by a frequency modulated sweep. Later work by Nobuo Suga's lab (see Volume 6) at Washington University showed that each component is represented in separate areas of auditory cortex. Neurons are tuned to distance-dependent echo delays and amplitudes relative to the bats own ultrasonic sound pulses.

In the 1970s, when I was graduate student at Rockefeller (see the next section), lunch was served at a long table in a room looking over the East River. If a student settled next to Don Griffin, he or she would be recruited for one of his projects: going aloft in a hot air balloon to record the vocalizations of migrating birds or using a "bat detector" to study bats emerging from under the roof tiles of the field station in Millbrook, New York. Don's wife, Jocelyn Crane, was an authority on fiddler crabs and our lab at Columbia inherited Joycelyn's tanks when she later turned to the study of art. The Griffins took me to dinner once at restaurant in the Hotel des Artistes below Jocelyn's apartment. They first retired to Princeton, New Jersey, and I house sat for them, luckily not when an ancient toaster set off a fire. Don and Joycelyn then moved to Massachusetts. Don kept a small sailboat and used it to work on the terminal buzz of *Myotis lucifigus* (the little brown bat) until he died. His admirable curiosity is well captured by the biographical sketch for National Academy of Sciences (NAS) by my Princeton University colleague, the late Charlie Gross (see Volume 6). I miss them both.

The Rockefeller University

Laboratory rotations, I believe, are as much about learning what you don't enjoy in research as what you do. My first laboratory rotation at Rockefeller was in Bruce McEwen's lab (see Volume 8). Bruce was a great mentor; not just when I was a student but throughout my career. He died unexpectedly last year (early 2020) to my great sorrow. My rotation project was characterizing corticosteroid receptor expression in the brains of baby hamsters in response to stress. After injection with tritiated steroid and then removing and homogenizing the brains, cellular components (membranes, cytoplasm, nuclei) were separated by high-speed centrifugation and the nuclear fraction was eluted from gel columns in the cold room. Bruce didn't have a fraction collector, so this meant moving scintillation vials by hand in sequence to receive the correct number of drops so that radioactivity could be measured in a counter after adding scintillation fluid.

I had nightmares about the baby hamsters. I was sure the centrifuge would blow up,. It was freezing in the cold room, and, more crucially, the data were all numbers on a paper strip. Where was the brain; where was the behavior? I did, however, learn enough steroid biochemistry to run studies when I had my own lab. I edited a volume of papers (Bruce put me up for the job) and presented my work in his lab at the first meeting of the SfN. The abstract (which I had forgotten) was followed 50 years later by an invitation from *Journal of Neuroscience* to review our research in an issue celebrating that first meeting. I greatly enjoyed writing this with many former trainees and current collaborators (Kelley et al. 2020).

Next, to try out electrophysiology, I examined the neural circuitry underlying the female rat lordosis reflex in Don Pfaff's lab. The idea was

to determine whether neurons in the central gray (PAG) were responsive to the sensory stimuli that induced lordosis: touch to the perineum. Surgery took the entire day, the recordings took the entire night, and I am an early morning person. Don then suggested a possible project that would fit my interests in sex determination and development. Emil Witschi had discovered that rearing *Xenopus* tadpoles in estradiol resulted in frogs that were 100% female. Backcrossing these frogs (treated as embryos) to untreated males resulted in sex ratios that revealed a zz (male), zw (female) primary sex determination system (i.e., some phenotypic females produced entirely male offspring). The experimental system seemed to be a promising one to determine whether there were any genetic contributions to sex differences in behavior or whether gonadal steroids (androgens and estrogens) were the sole determinants. This same question motivated the development of the "four core genotypes" in mice studied later by my fellow Grinnellian and Rockefeller graduate, Art Arnold, at the University of California, Los Angeles.

During my first year at Rockefeller I found out that many of my fellow RU graduate students (including Art) were going to Africa for a course on field studies of animal behavior. I asked to join them but they hadn't counted on including a psychologist. So, I applied to the Organization for Tropical Studies for a course on "Habitat exploitation and diversity; an ecological approach with vertebrates." My final rotation, during the summer of 1971, was thus field biology in Costa Rica. The course was organized by Roy McDiarmid (University of Florida) and Robert Rickliefs (University of Pennsylvania), a distinguished amphibian ecologist and ornithologist, respectively; visiting faculty included Jiro Kikawa from Queensland and Gordon Orians from Seattle. Among my fellow students were Sandy Vehrencamp, Frank Bonnacorso, Donna Howell, Marty Crump, Jim DeWeese, Ron Orenstein, and Dennis Turner. We started with Howler monkeys (and bats with harems for me) and then moved to the Osa Peninsula. Some highlights were mist netting in dry streambeds and finding the bottom panel full of vampire bats in the morning, the Jesus Christ lizard (*Basiliscus basiliscus*) running on top of a stream, gold Ponerine ants with painful bites, the huge *Vampyrum spectrum* that Donna caught flying around the mess hall, and the bat sea cave. Our course spanned the transition between wet and dry seasons, and when we returned to Guanacaste at the end of the course, my tree with the harem bat (*Saccopteryx bilineata*) was bat free. The advantages of laboratory research were clear to me. In my files, I find feedback on my lack of preparation in advanced theoretical ecology from Gordan Orians. Absolutely true; I was there mainly for the bats.

When I returned to Rockefeller, I started to explore the hormonal basis of reproductive behaviors in *Xenopus laevis*. Figuring out what was known about *Xenopus* required hours in the library stacks making notes on cards; lots to be said for Google Scholar and personal computers. However, I discovered the eccentric Sir Lancelot Hogben, the psychoanalytically inspired

studies of William Russell on *Xenopus* sex behaviors and John Hutchison's thesis research at the University of Capetown (UCT) on male clasping. I decided to start by determining whether the zz genotype contributed to the clasping "reflex" of males.

Mikamo, a scientist in Emil Witschi's group, then at the Population Council, gave me advice on rearing frogs. I started by replicating the estradiol effect with tadpoles. I'd induce females to lay eggs and males to fertilize them while clasping using human chorionic gonadotropin (the basis of the first pregnancy test). I set up the tanks, went away for the weekend and they had all died by Monday. It was the water (it is pretty much always the water with *Xenopus*). I next tried to feminize males with estradiol; they died too (too much estradiol is toxic to the liver). But when I ovariectomized females and implanted them with pellets of testosterone, they clasped other females. Clearly their zw genotype did not prevent them from exhibiting androgen-stimulated, male-typical sex behaviors. I turned from behavioral genetics to endocrinology and set about describing the effects of gonadal steroids on the behaviors of adults and mapping the distribution of androgen and estrogen receptors in their brains with Joan Morrell, now at Rutgers, but then a new postdoc in Don Pfaff's lab.

It was not until 2008 that the genetic underpinnings (i.e., DMW) of the *X. laevis* sex determination pathway were identified. How estrogen exposure during a short developmental window (tadpole stages 50–52) produces ovaries in genetic males, and whether or not estrogen plays an endogenous role in producing females, is not clear to this day. During this critical period, the developing gonad expresses ESR1 (estrogen receptor α) but not aromatase (the enzyme that converts testosterone or T to estradiol or E). However, the gonad is not the only possible source of estrogenic compounds in tadpoles, nor is estradiol the only estrogen. In 1995, before DMW was identified, Lydia Kang in our lab examined steroid synthesis in tadpole gonads and interrenal (frog adrenals) glands. Androgens (T and dihydrotestosterone or DHT) are detectable as early as tadpole stage 47. Δ5-3β-Dihydroxy steroid dehydrogenase, an essential enzyme for generating Δ5 steroids, is present in both interrenals and gonads at stage 47. Both DHT and T bind to the androgen receptor, but DHT metabolites, 3α and 3β diol, do not; the latter is a high-affinity ligand for ER2 (estrogen receptor β). My guess is that circulating 3βdiol from the interrenals binds to ER2 in the developing gonad and facilitates ovarian determination.

The genetic mechanisms responsible for primary sex determination vary across the *Xenopus* phylogeny; even within populations of one species (e.g., *X. tropicalis*; Roco et al. 2015). In contrast to the roles of estrogens and androgens, strongly conserved across vertebrates, primary sex determination is remarkably labile. All that matters is reliably producing offspring with ovaries and others with testes, although some genes (such as DMRT1) are particularly handy for the process.

Rockefeller University: Neural Control of Vocal Behavior

I defended my PhD in 1975. My papers (three research reports and a review) were already published. Don Griffin, who was on my committee, gently suggested that vocal behaviors might be more interesting than clasping. Art and Fred had just published a paper illustrating the pronounced sexual dimorphism of vocal control nuclei in the canary and zebra finch forebrain, so this experimental system seemed like a good one for a postdoc.

The goal of my postdoctoral research was to establish the connectivity of vocal control circuitry in the canary forebrain, starting with the thalamo-recipient nucleus, Field L, using two newly developed methods: anterograde tracing with tritiated amino acids and retrograde tracing with horseradish peroxidase (HRP). Bernice Grafstein (see Volume 3) and McEwen had just shown that radiolabeled amino acids are incorporated into proteins, transported from the cell body to axon terminals, released, and taken up by postsynaptic neurons. Jennifer and Matthew LeVail had shown that HRP is taken up by presynaptic endings and transported to the cell body. I determined how auditory input from a primary forebrain auditory nucleus (Field L) gains access to vocal motor circuitry (HVc and its target in ventral forebrain, RA). Some months into the project I asked to see the brain sections (Fink-Heimer staining to reveal degenerating terminals) that Teg Stokes and Tiana Leonard had prepared, outlining the location of Field L after lesions in the thalamic auditory nucleus, ovoidalis. To my horror I discovered that months of work had been based upon a single brain, which, however, turned out to have had a perfectly placed lesion. Tracing results appeared in two papers identifying new regions (Nif, Uva) in the song circuit. Coordinating what is heard with what is uttered, is one theme of our current research in *Xenopus*.

I had a marvelous time in grad school and as a postdoc. Lily Conrad, a very close friend to this day, joined Don Pfaff's lab in 1972 after undergrad research in Gary Lynch's lab at University of California, Irvine. Don describes Lily in one of his books as someone who could have been "the next Cajal" had she not gone off to med school. It was Harvard Medical School. Lily went into emergency medicine at the beginning of this specialty; her paper on cougar attacks is a classic. We'd hang out with the physics grad students, go disco dancing, take ballet and modern dance lessons, and run around at SfN meetings together.

Once I started looking for a job, about the middle of my second year as a postdoc, I'd share what I'd figured out with other friends, such as Tom Rainbow in the McEwen lab. I applied to two places, was invited to give a talk at both and chose the Princeton Psychology Department. I started the process of job negotiation, designing a lab, and writing a National Institutes of Health (NIH) grant while awaiting the birth of our daughter Danielle. Later, when I moved to Columbia, Tom and I collaborated on steroid

receptor biochemistry; we'd go out to play video games (PacMan, Centipede) during incubations. Tom taught me how to use my first personal computer, an Atari. One day at Columbia, in front of the mailboxes, my fellow faculty member, Steve Schuetze, asked me if I had heard the "news from Princeton." Tom had been killed jumping onto a train from Princeton to Philly (he was a faculty member at Penn). Steve, also a close friend, was himself killed by a truck during a bicycle trip to celebrate his promotion and tenure. "When sorrows come, they come not single spies, but in battalions" (Hamlet).

Mentors and Role Models

Becoming a faculty mentor is quite a lot like becoming a parent—or laboratory rotations—in the sense that it is as much about what you did not like—as a child or a trainee—as what you did. Today, when choices are wider, students can use the mentoring reputation and record of the advisor in addition to the science itself. At Rockefeller, when I was a graduate student and postdoctoral fellow, there were no women in tenure-track faculty ranks in my fields of interest. Don Pfaff, my PhD advisor, was a good model of a careful, methodical, productive, and thorough scientist. Fred Nottebohm, my postdoc advisor, was also very productive with an intellectual style characterized by imagination and metaphor.

No women faculty did not mean no women mentors. Christiana (Tiana) Leonard, for example, was a member of Carl Pfaffmann's group. Her MIT PhD advisor was Walle Nauta, a gifted neuroanatomist, and she trained me and Lily in histology and neuroanatomy. Tiana was also a major contributor to the early mapping of bird song system and subsequently had a distinguished career at the University of Florida. Bernice Grafstein, still an active professor at Weill-Cornell Medical School, was another mentor; she continued to teach developmental neurobiology to us even after she moved across the street to Cornell and is responsible for my enduring interest in the subject. I reconnected with her when I became a term trustee of the Grass Foundation. While Tiana and Bernice were clearly gifted researchers and role models, it did not appear, at that time, that Rockefeller would be the right environment for a young woman faculty member. Fortunately, time has wrought changes at Rockefeller, not just for women but for scientists from diverse backgrounds.

Princeton: Establishing a Model for Vocal Communication

When I interviewed for the job in psychology at Princeton, Mark Konishi (see Volume 6), who I'd also thought of for a postdoc along with Harvey Karten, then at Stony Brook), was on his way to CalTech. I asked him what he thought about the place, and he said that it was a good first job, and so it was. When I started, as is somehow usually the case, my lab wasn't ready,

so I commuted to teach and kept doing research at Rockefeller. I'd been hired, I surmised, to teach neuroanatomy and I prepared by sitting in on the Cornell Medical School course and making a slide set of the rat brain (mice had not yet ascended). I'd ferry frogs to and from New York City, usually by train. At PJ Clarke's restaurant one night, I met my mother for a dinner during which the people at the next table all stood up and fled. I figured it was a gigantic cockroach, but when I got home, two frogs were missing. I dashed back and scrabbled through the sawdust on the floor until the clean-up crew told me they were safe in a tub in the basement.

At Princeton, I also taught one lab course. My "students" were Dan Sanes, Terry Sejnowski, John Paton, and Marilyn Yodlowski. Louis Sokoloff's (see Volume 1) 2-deoxyglucose method had just come out (basically a precursor to the immediate early gene approach but based on trapping labeled metabolites of glucose in active neurons,) so I figured we'd try that out. Terry, then a postdoc in Alan Gelperin's lab, used the technique in a mollusk (*Limax*) and the rest of us applied it to *Xenopus*. The course resulted in four papers, including mine in *Science* (1980) and Terry's in *Nature* (1980). The first was a help in getting my second job. The second paper was also consequential, although perhaps not just for the science.

I was at a Gordon Conference that summer and arranged to meet Terry (who had just taken the neurobiology course at the MBL and was on his way to Steve Kuffler's lab at Harvard) in Woods Hole to revise the paper. I drove to Woods Hole and my strong ties to the MBL began (Kelley 2017). While writing Miranda Robertson (editor at the time; Dear Madam, so pompous of us), Terry and I decided to send in a possible cover picture of a 2DG-labeled *Limax* neuron. This same picture later appeared on the cover of a book on how life on Earth had been seeded by aliens. Don't ask; I have absolutely no idea. Anyway, Terry got Harvard to invite me for a seminar. and I met Alison Doupe, an extraordinary and creative bird song neuroscientist, then in Paul Patterson's lab. Alison was a wonderfully generous person and was also very funny. Mark Konishi (her postdoc advisor) once sent her in his stead to Paris to give a talk as she was fluent in French. Alison practiced the talk with her Parisian uncle and his partner. Their major comment was her Canadian accent; not something easily revised for a talk the next day. Alison and her lab were responsible for our understanding that two mysterious forebrain songbird nuclei, lMAN and area X, are homologs of the mammalian basal ganglia, leading to the idea that injecting variability into programs is an important circuit function for motor learning.

Why did I go back to *Xenopus* after my bird song postdoc? Mostly because I wanted to understand male- and female-specific innate vocal communication at the cellular and molecular levels. Also, birds are fragile, short-lived, and have small clutches; *Xenopus* is robust and long-lived and a single mating produces hundreds of tadpoles. Because *Xenopus* are nocturnal, totally aquatic throughout life and prefer murky ponds, sound production

and reception are essential for social communication. The songs themselves produce acoustical "proxies" for analysis of motor programs. Birds instead are diurnal and thus also use visual cues in social behavior. Their vocal organ is an evolutionary novelty, not an adaptation of the ancestral vocal organ shared by frogs and mammals: the larynx. Male canaries and zebra finches learn their songs, making them one of the few model systems for vocal learning, but I was more interested in sex differences in innate behaviors. Another consideration was independence. Nottebohm and Konishi had established a powerful model system for learned vocal behaviors; there was no reason I couldn't do the same for innate vocal behaviors, although I still maintain close ties to the bird song community.

At Princeton, our lab began to establish *Xenopus* as a model system for understanding vocal communication and its sexual differentiation. I wrapped up my thesis work by characterizing female sexual behavior, mapping DHT binding in the central nervous system (CNS), and (with Sol Erulkar's lab at Penn) showing that androgen controls the excitability of clasping motor neurons in the spinal cord. Dan Wetzel, our first graduate student (now a researcher at IBM), characterized male advertisement calling and its endocrine control, including an unexpected role for gonadotropin. Using the 2DG method, combined with steroid autoradiography, I showed that both auditory and vocal nuclei in *X. laevis* accumulate gonadal steroids. Martha Constantine-Paton, a frog developmental neurobiologist, then married to John Paton, was in the Princeton Biology Department. I had first met her as a graduate student in Bob Capranica's lab, swapping eye and ear anlagen in tadpoles. I planned to explore the developmental origins of sex differences in the vocal system; Martha helped me learn classic embryological transplantation methods at Princeton. On the train between New York City and Princeton, I'd spend the morning planning my lectures and the evenings marking up *Current Contents* for reprints.

Moving to Columbia

When I first visited the MBL (summer of 1980), Fred was teaching in the Neural Systems and Behavior course, as was Eduardo Macagno from Columbia. Ed had joined Cyrus Levinthal to convert Columbia's Zoology and Botany Departments into a single department: Biological Sciences. John Hildebrand had been recruited from Harvard. He shared my interests in developmental neurobiology as did Steve Schuetze, who had just arrived from Gerry Fischbach's lab (see Volume 9). Cy offered me a job, but I told him that I saw no point in starting over again but would move if Columbia offered me tenure. The result (after the usual to and fro, with some complications of departmental politics) was that I moved to Columbia in January of 1983 as a tenured faculty member, six and a half years after I was awarded the PhD. I thus was never subject to the excruciating experiences of even

the most accomplished junior faculty today and reduced my commute from 4 hours to 1.5 hours daily. I was also shielded from possible adverse consequences of being an outspoken person and never had to worry about antagonizing someone who might participate in the tenure decision.

As I reviewed my Princeton correspondence just before the move (there were secretaries who typed and made carbon copies, not emails), I remember the fall of 1981 as a busy and happy time with exchanges of paper reprints between our lab and many others. Marty Chalfie was also moving to Columbia from Sidney Brenner's lab and offered me a place to stay before that year's Neuroethology Gordon Research Conference in the United Kingdom. In 2008, I traveled to Stockholm when Marty was awarded the Nobel Prize and Martha Constantine-Paton, now at MIT and married to Bob Horvitz, was a fellow guest. I interviewed John and Martha's son Joe for Columbia's Neurobiology and Behavior (NB&B) graduate program without realizing that I'd published papers with both of his parents. Joe now directs the Champalimaud in Lisbon, and I advise their graduate program. Science is social.

When I joined Columbia's Biology Department, they had admitted a graduate student interested in our lab: David Sassoon. David (now at University of California, San Francisco, and the Salpetriere) pioneered our studies of sexually differentiated laryngeal development and its hormonal control and identified the juvenile muscle satellite cell (discovered by Alex Mauro at Rockefeller) as an androgen-responsive myogenic stem cell. Dennis Gorlick, our first postdoc, and Dan Wetzel also moved from Princeton to Columbia with me. Dan and Ursula Haerter (now a labor lawyer) mapped the *Xenopus* vocal circuit. Patty Hannigan (now Dr. Lotitio and in medical practice) continued her Princeton undergraduate thesis at Columbia, defining sensitive periods for masculinization of vocal behaviors.

I started teaching in NB&B and would take the entire lab to Woods Hole for the summer, including Rob Raguso (now at Cornell), a family friend of Patty's on his way to start college at Yale. Andy Bass, a PhD with Glenn Northcutt (now also at Cornell), joined us in teaching NB&B as did Neil Segil, a former lab member, now at USC. Russ Fernald (Stanford) set up a unit on the diurnal rhythm of photoreceptors in monkfish, collecting them with students in the morning, teaching the dissections after lectures, and cooking them for dinner. I was so happy that our children could be in Woods Hole during the summers, at Stony Beach, the Children's Science school, riding, swimming, playing tennis, and collecting insects. My MD/PhD husband Richard took the physiology course one summer and stood by to resuscitate us if needed when the collecting boat came up with a huge electric eel for Andy and Neil to dissect.

In 1982, I was also in conversation with Martha Tobias, a PhD student of Roy Ritzmann's at Case Western, to join the lab as a postdoc. John Hildebrand was directing the neurobiology course and Martha, who had

stayed on at the MBL to do research after the NS&B course was over, asked who at Columbia was working in neural development. We hadn't published anything yet, but John knew what was in the works and Martha joined us, rising through the ranks to senior research scientist.

Having Martha as a research partner was a boon; she brought with her not only a strong interest in behavior and development but also remarkable skills in electrophysiology. Until her (much-lamented) retirement, we ran the lab together. Martha was responsible for many early research findings, including the isolated vocal organ (larynx) that "sings" *ex vivo* and the role of estrogen in the strength of the vocal neuromuscular synapse. Although she did not share my interests in molecular biology and genetics, Martha became keenly interested in vocal evolution after Joe Thornton (now at Chicago) and Ben Evans (now at McMaster) joined us as graduate students and published two seminal papers on the evolution of vocal signaling in 2011 and 2014. Also in 1995, after Apartheid ended, Martha moved her family (the artists Richard and Luca Chiriani) to South Africa to start fieldwork on *Xenopus* (see the section "Field Research in Africa"). We later went to Gabon, advised by Carl Hopkins at Cornell, who was studying electric fish and had noticed *Xenopus* for sale in a market north of Libreville. The songs of the species we collected in Gabon (*X. mellotropicalis*) were included in Martha's 2011 paper, using the robust molecular and morphological phylogeny that Ben Evans and his lab at McMaster had established.

Sexual Differentiation

In the 1970s, our understanding of the basis for male- and female-specific ("sexually dimorphic") behaviors was based on a pivotal study in guinea pigs (Phoenix et al. 1959). The authors concluded that androgens act early in development to *organize* sexually differentiated behaviors and later in adulthood to *activate* behaviors. The questions we addressed in Xenopus in the 1980s were as follows: (1) How are these developmentally segregated effects exerted at the molecular and cellular levels? (2) What muscles and neural circuits are responsible? (3) How does the action of hormones sculpt and energize the neural circuits underlying sexually differentiated vocal behaviors in Xenopus? and (4) What are these circuits?

Answering these questions required characterizing the vocal repertoires of the sexes as well as their endocrine control, identifying sexually differentiated features that underlie the neuromuscular control of vocal behaviors and determining how these diverge developmentally in males and females. We discovered the responsible sensory and motor components, the neural circuitry that connects them as well as how each is shaped developmentally in the sexes and affected by hormones. For example, David Sassoon discovered that the greater number of laryngeal muscle fibers in males is due to androgen-stimulated proliferation of a specific class of muscle stem cells.

Neil Segil and David discovered the extraordinarily high levels of androgen binding in the developing larynx. John Roberts (now at Westminster College) discovered that thyroxine exposure during metamorphosis is required for testosterone-directed laryngeal masculinization (androgen competence). In the nervous system, I used the Golgi method to characterize the sexually differentiated dendritic arbors of adult vocal motor neurons and Jane Dennison from Australia used EM to find out how and when vocal motor neuron numbers diverged in the sexes. I also carried out my first molecular biology experiments in 1985 to identify tissue-specific expression of mRNAs in *Xenopus* tadpoles with fellow faculty Mark and Eva Rastl-Dworkin.

The 1980s were a prime period for developmental neurobiology. We learned, for example, that in *Xenopus* androgens can regulate ontogenetic cell death to sculpt sex differences in laryngeal motor neuron numbers. As an undergraduate, Jeremy Kay (now at Duke) identified the dying laryngeal motor neurons. The complex crosstalk between these neurons, their muscle targets, and androgens was clarified by Melanie Marin (now at Mt. Sinai) and Martha. Chris Edwards determined that prolactin closes the sensitive period for androgen action on laryngeal motor neurons and their muscle targets. Chris went on to postdoc with Gary Rose and discovered the fascinating neurons in the frog inferior colliculus that count sound pulses; he is now in medical practice.

In short, sexual differentiation of the effectors for male-specific vocal patterns is due to orchestrated action of hormones that determine timing of cell proliferation, differentiation, and cell death. In many ways, *Xenopus*—with its great experimental advantages for developmental biology and striking sex differences in vocal behaviors and their neural underpinnings as well as sensitivity to the same hormones that direct sexual differentiation across vertebrates—has been a powerful model system for understanding the hormonal control of sex differences during development.

Vox ex Vivo: The Isolated Brain and Larynx as Engines for Discovery

At Columbia, we also began to explore how the brain and vocal organs generate vocalizations in males and females. In the summer of 1984, Martha and I stayed on at the MBL after the neural systems course ended to try to coax isolated brains to "sing"—that is, to generate patterns of activity recorded from the laryngeal nerve as it exits the brain that match patterns of male- and female-specific songs (known as "fictive" songs). When these attempts failed, Martha turned to the larynx, stimulating the laryngeal motor nerve as it enters the brain posteriorly. If the nerve was stimulated with the pattern of a male's song, the attached larynx generated actual sound pulses that matched those produced by a frog singing under water, including the sound frequencies that make up each sound: *vox ex vivo*. As a postdoc, Ayako Yamaguchi (now at Utah) recorded from the laryngeal nerve of singing male

and female frogs and showed that nerve compound action potential (CAP) patterns matched male- and female-specific actual vocal patterns. CAP shapes in males (but not females) closely resemble individual motor neuron action potentials, suggesting that male laryngeal motor neuron firing is closely coordinated. Martha then used the isolated larynx to discover (using a classic quantal analysis) that laryngeal motor neuron synapses (NMJs) in females are "strong": each nerve action potential releases enough acetylcholine for the muscle fiber to depolarize and produce an action potential, whereas male synapses are "weak," requiring multiple action potentials for muscle fiber contraction, a sex difference I have always relished. We then went on to show that the synapse starts out during development as weak in both sexes and strengthens in females during exposure to estrogen during puberty. Exactly how this happens at the molecular level is still not known, though Kwok Hang Wu (now at Yiling Pharmaceutical) in his postdoctoral research, uncovered several estrogen nuclear receptor variants that are candidate participants in this process. Together these studies revealed that, in *X. laevis*, male- and female-specific vocal patterns are generated by the brain and that the male- and female-specific spectral features of vocalizations are due to the larynx. This separation of functions turned out to be critical for our current genomic approaches.

Refining the medium bathing the frog brain and continuously oxygenating this "frog juice" allowed us to use the *ex vivo* preparations to understand the neural circuitry that generates song patterns. With Blair Simpson (an NS&B student who joined our lab briefly after 1984 NS&B; now professor of psychiatry at Columbia), we used the isolated brain to map the locations of glottal and laryngeal motor neurons by applying the fluorescent dye lucifer yellow to each of the nerve rootlets that form the N.IX-X bundle as it exits the brain case. She then double-labeled laryngeal motor neurons by injecting HRP into laryngeal muscles, revealing sexual dimorphisms in number, location, and size of vocal motor neurons. Later, an undergraduate at Columbia, Catherine Brahic (now environment editor at the *Economist*), confirmed and extended our previous in vivo studies using HRP-WGA in vivo. She used anterograde and retrograde tracing in the *ex vivo* brain and discovered the involvement of the dorsal raphe nucleus in hindbrain vocal circuitry. All of the connections Catherine described are present in both male and female brains (though more robust in males) but must function differently in response to the sex-specific hormonal environment.

The motor neurons that drive sound production are located at the caudal end of nucleus ambiguus (NA) in the hindbrain. As a graduate student, Erik Zornik (now at Reed) injected fluorescent tracers into NA and its major afferent, the parabrachial nucleus (PB) in *ex vivo* brains, establishing the interneuron classes that account for the ipsi- and contra-lateral connection in the hindbrain vocal circuit. Ayako Yamaguchi (now at the University of Utah and Erik's postdoc advisor) followed up on this observation when she

established her lab at Boston University, showing that 13 weeks of androgen treatment can completely masculinize vocalizations of adult females. With Heather Rhodes (now at Dennison), Ayako found that when an isolated *Xenopus* brain is exposed to serotonin, fictive male- and female-specific "songs" can be recorded from the laryngeal nerve as it exits the hindbrain. This effect of exogenous serotonin reflects the endogenous action of serotonergic neurons in the raphe on the vocal circuit described by Catherine Brahic.

One challenge in going from hormonal control of behavior to underlying mechanisms is determining which hormone targets (sensory, motor, CNS) are responsible for endocrine actions. The *ex vivo* approach allowed us to define the effects of hormones on multiple components of the vocal circuit. As a graduate student in our lab, Erik Zornik used the *ex vivo* brain to establish that PB, formerly known as DTAM), provides powerful, monosynaptic, excitatory input to laryngeal motor neurons in caudal NA while inhibiting glottal motor neurons in rostral NA. This circuit motif is essential for vocal production in *Xenopus* because the closed glottis prevents fluids in the buccal cavity from entering the space above the arytenoid disks, which (as I showed later with Coen Elemans at Southern Denmark University or SDU) prevents disk separation from attaining the velocity required for sound production.

The nuclei of the hindbrain are highly conserved across extant vertebrates, from frogs to mammals. The parabrachial complex in mammals includes neurons that gate the expiratory to inspiratory transition. Air-powered sound production requires an open glottis. Maintaining the ability to produce courtship vocalizations during the evolutionary transition from land back to water in *Xenopus* thus required a switch in PB neuron connectivity from excitatory to inhibitory. What drives the activity of PB neurons? In her PhD thesis, Irene Ballagh (now at the University of British Columbia) used microstimulation and dye-tracing in *ex vivo* male and female brains to establish reciprocal, functional connectivity between PB and the central nucleus of the amygdala (CeA). Fictive sex-specific songs can be elicited *ex vivo* by microstimulation of the CeA in both sexes. In mammals (mice, bats, and rhesus monkeys) the CeA also responds to, and is active during, the production of vocal signals.,

The *ex vivo* brain also revealed novel endocrine contributions to the *Xenopus* song circuit. Dan Wetzel's PhD thesis had shown that male advertisement calling relies on gonadal androgen and that a pituitary hormone, gonadotropin (luteinizing hormone, LH) synergizes with androgen to enable robust male calling behavior. As a postdoc, Eun-Jin Yang (now at GlaxoSmithKline) and graduate student Brian Nasipak (now at Alnyam Pharmaceuticals) discovered that gonadotropin, acting through the LH receptor, acts directly on the CNS to increase calling. LH application to the *ex vivo Xenopus* brain increases expression of its target, egr-1, even in the

absence of neural transmission. Gonadotropin thus may play a novel integrative role in modulating both reproductive physiology (modulating circulating androgen or estrogen levels) and behaviors

Field Research in Africa

Xenopus are species of the family Pipidae, native to Africa. Pipids are Paleobatrachian frogs that migrated from what is now Europe into Gondwanaland (then the conjoined continents of Africa and South America) approximately 160 million years ago. Other Pipids are found in South and Central America and all are aquatic. The Pipids survived the mass extinction event at the Cretaceous-Paleocene boundary that wiped out many terrestrial frog species, leading to the rapid speciation of the more recently evolved *Neobatrchia* species threatened today by global climate change and habitat loss. I suspect that when we humans are long gone from this planet (via migration to other worlds and/or extinction), if Earth still supports its animal life forms, *Xenopus* will be among them.

To get at the actual biology of our lab findings in *Xenopus*, we traveled to Africa. When Apartheid ended in 1995, Martha and her family moved to Capetown in South Africa to study the species' vocal communication. Luca enrolled in a Rudolf Steiner school where he never had to wear shoes, Richard painted, and Martha was hosted by Mike Picker at UCT, who also had hosted Ben Evans's research on hybrid zones between *X. gilli* and *X. laevis* a few years before. Martha's family settled into a house in Rondebosch that (crucially) had a swimming pool and into life in immediately post-Apartheid South Africa, with its multiple curious aspects that I also experienced when I visited.

Usually, research in neuroethology starts from behavior and then moves to neural circuits. In our case, however, this direction was reversed. A male *X. laevis'* advertisement call contains alternating rapid and slow trains of sound pulses (fast trill and slow trill). Fast trill is amplitude modulated: the loudness of each sound pulse increases progressively within each fast trill segment. Male-specific facilitation at the laryngeal neuromuscular synapses (Martha) and progressive recruitment of motor neurons (described by Ayako Yamaguchi during her postdoc) account for the progressive loudness of sound pulses during fast trill. Female laryngeal synapses do not facilitate. Martha used quantal analysis to confirm that the locus of this sex difference was pre- rather than postsynaptic. The male larynx can only produce sound pulses because each successive action potential increases the amount of acetylcholine released from the synapse.

In the lab, females are more attracted to intensity-modulated male songs than to calls without this feature. Modulating the intensity of a behavior used during social interactions is a common motif in ethology (animal behavior). I wondered whether, for example, this modulation helped the

female find the singing male underwater when she was just about to extrude her eggs. You might ask, why did we have to go to Africa to find this out? We had tried phonotaxis (attraction to sound) behavioral experiments in the lab using Jocelyn Crane's crab tanks. A rotating graduate student spent an entire summer broadcasting calls that varied in amplitude modulation to sexually receptive females from an underwater loudspeaker. He discovered that a fiberglass tank created acoustic "nulls" as the female swam toward the male (the reflected sound waves cancelled out the frog's actual song) and so returned to medical school. We eventually built a large trapezoidal tank that had better acoustics for recording male songs across species. Recently, Ursula Kwong-Brown and Young Mi Kwon in our lab found that a large glass aquarium works best because the sound energy radiates into the air outside the tank in response to sound pulse impact and echoes are greatly diminished. However, our "field research" study was in 1995 not in 2019 to 2022.

The swimming pool in Rondebosch (termed the "artificial pond" in our 1998 *PNAS* paper) turned out to have excellent acoustics. Martha's approach was to slow down the male's ability to locate the female by confining her to the end of the pool, where the steps were, with a plywood barrier. A funnel trap inserted into a hole in the middle of the barrier let the male enter the female's chamber but not exit. However, wet plywood is acoustically transparent. The male could not find the hole and began swimming rapidly back and forth along the length of the barrier, producing advertisement calls. The sexually receptive female responded to the vocalizing male with a call we had never heard before that Martha named "rapping" (it sounds like a Geiger counter or machine-gun fire). The male then called back with an ardent version of advertisement calling, the answer call, creating underwater duets. Males do not habituate to rapping; it is an acoustic aphrodisiac. In contrast, Taffeta Elliot (now at New Mexico Tech) showed in her PhD thesis that female ticking suppresses male calling.

In ponds that contained hundreds of sexually mature males, Martha would record only one calling male. Back in the lab, she determined that this observation reflects male vocal dominance. Pair-wise testing revealed that dominance hierarchies last for up to two weeks. Groups of terrestrial male frogs form calling choruses. Females can locate males because in air call intensity diminishes rapidly with distance. In water, however, sounds can be heard over very long distances. Male-male vocal suppression avoids underwater cacophony that would hinder the female's ability to locate a calling male. Testing pairs of males revealed that male-male vocal dominance is long-lasting (at least 45 minutes). Taffeta, however, found that the suppression of male calling by the female unreceptive call (ticking) is transitory. Males habituate after about 15 minutes and go back to calling. So, the socially appropriate response of a male-to-female rapping is to switch from advertisement to answer calling. The appropriate response to male ticking

is transient vocal suppression, while the response to advertisement calling should be prolonged vocal suppression. These observations later enabled Ian Hall to determine, using lesions, that the extended amygdala (CeA/BNST) is essential for a male's ability to produce socially appropriate vocal responses to ticking vs. rapping calls from females.

Back in the lab, Sandya Viswanathan during her undergraduate (USC) summer research helped us replicate and extend the Rondebosch results. Because the mechanism of sound production in *Xenopus* is internal (rapid separation of the arytenoid disks producing vibration of the whole body), visual inspection doesn't tell you which frog in a pair is calling. However, the frequencies that make up sound pulses in males and females differ, allowing male and female contributions to the duets to be readily distinguished. For male-male pairs, we used an insensitive hydrophone (a waterproofed hearing aid) to distinguish caller from listener. Vocal responses can also be reliably evoked by underwater call broadcasts. Taffeta Elliott presented males with broadcasts of female calls in which the interval between sound pulses varied from short (rapping) to long (ticking). From the male's response (transient vocal suppression or answer calling), she determined that the interpulse interval border between ticking and rapping is graded rather than categorical. Playbacks also allowed us to disentangle the respective roles of spectral (pitch) and temporal (rhythm) features in vocal communication. As a postdoc, Clementine Vignal (now at the Sorbonne) then determined that males use the frequency content (spectral features) of sound pulses to identify the sex of the caller and the rate of sound pulses (temporal features) to distinguish attractive from repulsive female calls.

Neural Circuits for Vocal Communication

Understanding the neural link between production and perception of vocal signals requires knowing where auditory information intersects with pathways that control vocal patterns. Chris Edwards mapped the hindbrain and midbrain components of the ascending auditory pathway and Taffeta (coadvised by Jakob Christensen-Dalsgaard at SDU) recorded from the *Xenopus* inferior colliculus, determining that neurons in the laminar nucleus are rate tuned. At the time, we had not yet discovered the phylogenetic significance of the two dominant frequencies (dyads) that comprise male sound pulses in *Xenopus*, so the spectral representation of dyads from the auditory midbrain to the forebrain remains to be explored.

Many years earlier, Aubrey Gorbman had speculated that a region of the forebrain, the ventral striatum (VS), might receive auditory information. When Catherine Brahic was in our lab, she placed fluororuby into the parabrachial nucleus in the *ex vivo* brain and showed that VS and PB are reciprocally connected. Studies of transcription factor expression by Spanish neuroanatomists (Morona and Gonzalez, 2013) then revealed that

the VS is homologous to the central nucleus of the mammalian amygdala (CeA). When Ian Hall (now at Benedictine) lesioned the CeA in vivo, males lost their ability to distinguish information on sex and reproductive state conveyed by calls. Males responded to rapping with vocal suppression (the appropriate response to a dominant male) rather than with the answer call (the appropriate response to an ovipositing female). How this acoustic information is represented within the CeA remains to be discovered. However, the highly evolutionarily conserved CeA is clearly a key hub for recognizing social cues conveyed by conspecific sounds (across species, including mice, bats, and nonhuman primates). Ian and Irene's experimental results form the basis for our current explorations in *Xenopus* of human neurodevelopmental disorders that affect social interactions.

Evolution of Vocal Circuits

Anyone exploring the biology of courtship, sex differences in behavior, neural circuits for innate behaviors, and related subjects will, sooner or later, be drawn to Darwin's ideas on sexual selection. I started reading *The Descent of Man* on my train trips back from Princeton, perhaps influenced by colleagues in biology: Bob May and the Grants (Rosemary and Peter). Darwin's convictions about the intellectual differences between men and women were irritating but provoked the idea that the expression of receptors responsive to sex-specific patterns of hormone exposure could be an important underlying mechanism for sex differences.

The evolution of neural circuits for producing and responding to innate (species-specific) vocalizations is a subject of our current research. Ursula Kwong-Brown, then a Columbia College Music and Neuroscience major, working with Martha, made a key discovery. Looking up from the bench one day while the underwater songs of males from various species were being played, Ursula asked: "Why is that frog singing a perfect fourth?" You can listen to what she heard online (Kelley and Kwong-Brown 2019).

Ursula and Martha discovered that the two dominant frequencies (DFs; i.e., dyads) in the sound pulses of male advertisement calls in each *Xenopus* "subgroup" (A, M, and L) form a musical interval: a perfect fourth (A), an octave (M), or a major/minor third (L). The exception is *X. laevis*, the species we had studied for many years, in which the DF2 (higher) to DF1 (lower) ratio is 1.14 instead of 1.22. This difference in sound dyad ratios between species that can hybridize (produce fertile progeny) now allows us to identify genetic loci associated with species differences in laryngeal sound production.

Ursula, Martha, and I then traveled to the University of Massachusetts, Amherst, to visit Sheila Patek (now at Duke), an expert in animal sound production mechanisms. Sheila pointed out that acoustic properties of *Xenopus* sound pulses could simply be an artifact of recording in glass tanks.

For example, the snaps of the snapping shrimp in her lab weren't "snappy" when recorded in the field (the great barrier reef in Australia); they were "thuddy." We knew from Martha's research that the sound frequencies in a pulse recorded from a male *X. laevis* in "the dish" (*vox ex vivo*) were the same as recordings in tanks (*vox in vivo*). I had just heard a talk by Ron Hoy about Damian Elias' studies of the courtship "songs" of spiders: web vibrations that could be analyzed by shining light from a laser at webs and measuring reflected wavelength's Doppler shifts. The returning reflection is higher when the web moves toward the laser source and lower when the web moves away. This is the same idea as the Doppler shift used by echolocating bats to determine whether the flying insect is going away from or coming toward them. I called Ron and he suggested contacting Damian. We called Damian and he hopped on a plane from Berkeley bringing his field laser with him.

The laser and sound recordings from a frog singing in a glass tank matched the acoustic recordings *in vivo* and *in vitro* across species. In fact, all parts of a frog's body vibrate as a dyad when he sings; Ursula could record dyads from a single toe. These observations were key to figuring out (with Coen Elemans at SDU) how *Xenopus* produce their songs underwater without using vibration of laryngeal membranes powered by expiration. Air-powered vocal production is the mechanism we humans use and is one shared with current terrestrial frogs as well as ancestral *Xenopus* before they ventured back from land to water. Laryngeal vibration that produces two sound frequencies is an evolutionary adaptation that enabled *Xenopus* to continue to use vocal communication during courtship underwater.

Genetic Underpinnings of Innate Songs

While I was at Princeton, Christine ("Janni") Nusslein-Volhard gave a lecture on the genetic control of development in fruit flies. When we talked, she revealed that she had wanted to determine the genetic underpinnings of fly innate behaviors but switched to development as more tractable. Later, at a Genes and Behavior Gordon Conference, I heard Kerry Shaw (Cornell) describe her investigations of courtship songs in Hawaiian crickets using a quantitative trait loci (QTL) approach, eventually revealing a close linkage between the courtship signals of males and the acoustic preferences of females.

Shaw's studies illustrate one of the major challenges for understanding the evolution of vocal communication in courtship. How, as species and their courtship songs diverge, does the perception of that song diverge in concert? While we usually think of males doing the singing and females the listening, conspecific males also interact vocally (e.g., choruses); how do they recognize their vocal partners or adversaries? What about female duets?

Martha showed that *X. laevis* females are not vocal when together, although they do compete for a male when gravid. I suspect that in species in which access to males poses a challenge to reproductive success, we might find females that also compete vocally.

When I met David Kingsley (development and evolution of spines in sticklebacks), he pointed out that the major challenge in using QTLs to identify genomic loci associated with a particular trait is *quantitatively* phenotyping that trait. However, Martha's evolutionary studies of males and female *Xenopus* vocalizations had done just that, establishing temporal and spectral characterizations of vocal signals and their variation across the *Xenopus* phylogeny. So, with advice from Ben Evans, I began to think about how we might approach the genetic basis for species differences in *Xenopus* song production and perception.

One catalyst was Charlotte Barkan's PhD thesis in our lab, which explored characteristics of male songs in two related species: *X. laevis* from South Africa and *X. petersii* from Congo. Hybrids between the two species produce intermediate vocal patterns in males. When Andres Bendesky joined the Columbia faculty, I heard him describe his postdoctoral research with Young Mi Kwon in Hopi Hoeckstra's lab at Harvard. They used F2 hybrids between two species of *Peromyscus* (biparental and uniparental) to identify genomic loci associated with male and female parental behaviors. For this challenging project, Andres developed quantitative measures of parenting in both sexes. Using a hidden Markov model and low coverage sequencing, he correlated parenting styles and maternal or paternal species-specific genomic loci to identify candidate genes associated with these innate behavioral traits. Another catalyst was Yun Ding and David Stern's (Janelia) 2016 *Nature* paper identifying an intronic retroelement in a calcium-activated potassium channel gene associated with interspecific variation in the frequency of sine song in flies. The "power of Drosophila genetics" (yes, I have species envy) pointed to a genetic change affecting sine song frequency. While female preference actually relies on the pulse part of the male's biphasic songs rather than the sine part, this paper provided important proof of principle that the genetic approach to understanding the divergence of vocal behavior during speciation could work.

Xenopus has some real advantages for dissecting the genetic bases for innate differences in vocal communication. Studies in *ex vivo* preparations (the isolated larynx and brain) clearly revealed that the spectral properties of male songs are inherent to the vocal organ, while the temporal properties are inherent to the brain. This is not the case for other vocalizing species, such as terrestrial frogs, birds, and mammals, in which air movement and vibrating membranes power sound production, and both are driven by CNS motor neurons. Spectral features in birds/primates also require coordination with the syrinx/vocal tract or larynx/vocal tract and thus also rely on the CNS. In contrast, frequency information in *Xenopus* sound pulses is entirely

due to the properties of its vocal instrument, the larynx. Temporal information in calls is almost entirely due to the hindbrain vocal pattern generator. In both sexes, laryngeal motor neurons and muscles must contract sufficiently rapidly to separate the arytenoid discs and cause the larynx (and the whole body) to vibrate, generating sound pulses underwater.

Martha had shown in her 2011 paper that the calls of both *X. petersii* and *X. laevis* are biphasic: periods of fast trill alternate with slow trill. By recording from the vocal nerve in the fictively singing brain of male *X. laevis*, together with recording from the PB in the rostral hindbrain, Ayako's laboratory found that a distinctive local field potential (LFP) accompanies fast trill. Bob Schmidt had also shown much earlier that this LFP could be recorded from the PB in terrestrial frogs; thus, PB is a candidate ancestral character for anuran vocal production. In her thesis research (coadvised by Erik Zornik), Charlotte Barkan found a PB LFP in *X. petersii* with a duration corresponding to fast trill. The PB includes two types of rhythmically active neurons: fast trill neurons (FTNs) and early vocal neurons (EVNs). When synaptically isolated and stimulated with NMDA, the membrane potential of FTNs (but not EVNs) oscillates at the species-specific duration and rhythm. FTNs are thus strong cellular candidates for species-specific genetic differences that drive innate song patterns. An extensive pharmacological survey has not yet revealed specific cellular or molecular candidates for the species-specificity of FTN membrane oscillations. Our current QTL approach focusing on temporal aspects of advertisement calls should help to reveal candidate molecular mechanisms for heritable song patterns (CNS) as well as dyad harmonics (vocal organ).

X. laevis and *X. petersii* male songs alternate between fast and slow trills. *D. melanogaster* male songs are also biphasic; they alternate between pulse and sine song. So, when Emilie Perez and Avelyne Villain joined our lab as postdocs after their PhDs with Clementine Vignal, we asked Christa Baker and Mala Murthy at Princeton to collaborate in developing ways to analyze the quantitative variations in call features that we expected to find in *X. laevis*/*X. petersii* hybrids. Emilie, Avelyne, and Christa developed analytical methods that demonstrated variability in the spectral and temporal characteristics of F1 male hybrid songs. Andres and I recruited Young Mi Kwon back to the United States from Cambridge in the United Kingdom, and she then developed an automated pipeline for acoustic analyses for the many hundreds of male F2 hybrids required for the QTL analysis. Stay tuned, but we are cautiously optimistic that genomic loci associated with species-specific temporal features will include genes that contribute to innate vocal rhythms. Similarly, biomechanical and/or morphological features of the larynx also should be associated with the genetic divergence responsible for species-specific dyad ratios.

What about matching production to perception? While in our lab as a postdoc, Clementine Vignal had shown that *Xenopus* can recognize

sex-specific spectral features of songs. Does auditory recognition of spectral or temporal vocal features coevolve with speciation? When Ian Hall joined our lab as a postdoc, Martha and Ursula had started developing auditory evoked potentials (AEPs) to look at auditory sensitivity to spectral features with help from Bob Dooling's lab and Catherine Carr at the University of Maryland. Ian Hall then used AEPs to show that the auditory sensitivity of females was greatest, across several *Xenopus* species, for the spectral features of their own species' male songs. Shifting either the higher frequency (DF2) or the lower frequency (DF1) in a sound pulse by a few hundred Hz abolishes this preferential sensitivity. This acoustic advantage is present in females but not in males and depends on gonadal steroids (testosterone is the major circulating hormone of females). AEPs most likely reflect activity in the acoustic ganglion and perhaps the dorsal acoustic medulla. I had shown that neurons in the *X. laevis* acoustic ganglion accumulate DHT (an androgenic precursor to 3β-diol), providing a possible explanation for hormonal regulation of auditory sensitivity. Emilie Perez then used AEPs to examine the auditory sensitivity of female F1 hybrids and found them to be most sensitive to the frequencies in male hybrid sound pulses compared with either parental species. One goal of our current studies in F2 *X. laevis*/*X. petersii* hybrids is to determine whether this sensitivity confers a behavioral advantage for females in locating a genetically compatible male.

I've glossed over the major experimental disadvantage of *Xenopus* for QTL analyses: the long time to reach sexual maturity and adult song production. Although careful attention to rearing can accelerate development, male *X. laevis* typically take at least 6 months (and females a year) before exhibiting an adult repertoire of behaviors. So, while a single mating can generate the large numbers of animals needed for statistical analyses of QTL data, we have had to wait for the F2s to grow up. Ironically, the 2020 Covid research pause was an advantage. Before the pause, we collected promising preliminary QTL data on male song spectral features. We are now analyzing temporal F2 hybrid vocal features in both sexes and their association with genomic fragments of maternal (*petersii*) or paternal (*laevis*) origin. When I first described this project to Mala Murthy, she commented that it is exceptionally ambitious. My suspicion is that she meant: "for someone whose experimental animals might outlive her." I do have 35 year-old *Xenopus* in our colony. "If not now, when?" I replied.

Behavioral Genetics

One of the most contentious aspects of research in behavioral genetics is the idea that there are "genes for a behavior." Many human behaviors are learned (e.g., language), and the idea that the individual's genetic background might make a major contribution to their behavioral repertoire is controversial, to put it mildly. We do accept, however, that many human

disorders are strongly influenced by an individual's genome. For example, mutations in the BRACA1 and 2 genes have strong associations with breast cancer. Women with these genetic variants can choose to have prophylactic mastectomies. Some communities require testing for Tay-Sachs disease before a couple's first date, identifying the risk that falling in love would have for future children. The brain is also an organ and subject to genetic variation, especially during development. Genetic variants that shape neural circuits have the potential to profoundly influence behaviors, including social behaviors and cognition, for better or for worse, and sometimes for both. For example, an individual's genome might contribute to an intellectual disability or speech alterations or instead to enhanced sociality or creativity. Bipolar disorder (manic depression)—the most heritable of the neuropsychiatric disorders—is characterized by periodic alterations in mood but also by epochs of heightened creativity, as Kay Jamieson has so vividly described in her books.

After my initial exposure to autism (now termed autism spectrum disorder or ASD) as a high school student at the NSF summer science institute in Iowa, I was introduced again to behavioral genetics and neuropsychiatry when Ed Kravitz (see Volume 4) asked me to join the scientific advisory board of the Hereditary Disease Foundation (HDF), which is where I met Anne Young (see Volume 8). Founded by Milton Wexler and his daughter Nancy, the HDF is focused on Huntington's disease (HD) but with the underlying premise that many neuropsychiatric diseases treated by Milton in his practice also were subject to inherited genetic variants.

My first meeting was in Santa Monica. The HD gene (Huntingin) had not yet been identified. The session opened with the presentation of a patient and was followed by a brainstorming session on how to identify the gene and ultimately understand how its known effects, the death of medium spiny neurons in the striatum, contribute to motor phenotypes (chorea) as well as to emotional and cognitive phenotypes. This simultaneously fascinating and catastrophic disease was ultimately traced to the Huntingtin gene and its expanded trinucleotide repeats; some progress in treatment has begun. My experience with the HDF, and that early exposure in Iowa to Nate Azrin and Ogden Lindsley's attempts to use operant conditioning to shape the behaviors of autistic children, contributed to my current interests in the biology of neuropsychiatric disorders described next. The other contributing motivation came from realizing that my grandmother's long illness was the result of severe bipolar disorder that must have genetic antecedents, although what these are, and how they relate to her psychosis, are not yet clear.

My thinking about genetics and behavior was also influenced by a workshop on the "Neuroscience Workforce" at the National Institute of Medicine, attended by representatives of "big Pharma." The research programs at leading companies had switched from neural diseases, such as Alzheimer's,

to cancer because the biology was much better understood. Most neuropsychiatric disorders have a characteristic developmental trajectory. How can neuroscientists understand the basic biology of complex, genetically influenced behavioral variants and their severity?

While I was growing up, autism (see *Family Pictures* by Sue Miller), was viewed as the result of maternal behaviors: the cold mother. While behavioral therapies could be effective, particularly for self-harm, the psychoanalytic viewpoint prevailed for many years. Today, efforts by individual researchers and foundations (e.g., Simons) have enabled the identification of rare, highly penetrant genetic variants associated with ASD. Figuring out how these genes contribute to disease is a focus of many laboratories using mouse models and organoids.

The problem as I see it, however, is the basic biology. Autism is a neurodevelopmental disorder sometimes associated with intellectual disability and sometimes with specialized intellectual abilities (or both). A common feature across ASD is difficulty in social interactions. Would it be possible to understand the biological consequences of neuropsychiatric disorders and their associated genetic variants in a model system in which neural development is accessible, in which social behaviors and their development are well-characterized and quantifiable, and for which underlying neural circuits have been identified? That would be *Xenopus*.

Autistic Frogs?

We currently are gearing up to determine if knockouts of rare, highly penetrant ASD genes in *Xenopus* produce behavioral phenotypes, and if these can be rescued by exposure to estrogen during development. The inspiration for this project comes directly from recent work by Helen Willsey on *X. tropicalis* (Willsey et al., 2021) in which CRISPR knockouts of rare, highly penetrant ASD candidate genes in one cell of the two-cell-stage embryo produce either a larger or smaller forebrain in tadpoles. These morphological phenotypes can be rescued by drugs in the estrogen signaling pathway. Was Despert's 1971 paper prescient?

I concede that frogs are not great models for the intellectual disabilities that often accompany early onset neuropsychiatric disorders. However, we have characterized the behavioral repertoire of both sexes in *Xenopus* during courtship and mating, and vocal components are readily quantified. From Gurdon's early work growing tadpoles from eggs with nuclei from somatic cells to the present, *Xenopus* has been a powerful model for developmental biology. Mouse models currently dominate explorations of human developmental disorders, despite the inconvenience of interuterine development, in part because of major investment by the NIH in creating transgenic mouse lines and multiple experimental tools (e.g., CRISPR, viral vectors). However, as more and more genetic variants associated with

human disease are identified, and as these experimental tools are applied to *Xenopus*, this animal model has emerged as a powerful biological system for understanding the basic biology of genetically influenced human disorders. We are simply extending this approach to innate social behaviors and their underlying neural circuitry.

Large stretches of the human genome are devoid of gene ontology (annotation linking genotype to phenotype). Families faced with a variant identified as contributing to the illness of their child, a variant whose "function" is not known, can recognize (through studies in animal models) that the variant is a "real thing." It has a biological basis rather than resulting from their parenting styles or maternal factors. The social behavior network in the brain is one of the most evolutionarily conserved circuits in vertebrates. For example, the central nucleus of the amygdala—known to contribute to the production and perception of vocal signals during social behaviors from bats to monkeys—is essential for *Xenopus* males to distinguish the "voice" (male or female) of a singing frog and respond with socially appropriate vocalization (Hall, Ballagh, and Kelley 2013). The ability to make this distinction is required for reproductive success, plays a role in speciation and must be strongly selected for over evolutionary time scales. Autistic frogs? Maybe not, but genes and circuitry and behavioral alterations, probably yes. We will see.

Educating Scientists

Undergraduate Education

At Princeton, my first teaching assignment was developmental psychology. This was not a course I had ever taken. When I asked: "Why me?" I was told: "Well, you have children, don't you?" I asked Marc Bornstein—who had taught this course the previous year but then moved to NYU—what I should cover. He said that it didn't really matter so long as I gave one lecture on Freud and one on Piaget. I followed his advice; the remaining lectures were on the development of behaviors in species other than our own. Incorporating Piaget and Freud was easier than I imagined, in part because both had started out as biologists: Piaget studying the shells of mollusks and Freud search for the elusive reproductive organs of eels. As Piaget said: "I am convinced that there is no sort of boundary between the living and the mental or between the biological and the psychological. From the moment an organism takes account of a previous experience and adapts to a new situation, that very much resembles psychology" (Bringuier, 1969). I covered bird song as a model for children's language development, as well as the seminal studies of Patricia Kuhl and the near-at-hand developing speech of our own children. My graduate teaching at Princeton was focused on neuroanatomy, a foundational skill for neuroscientists.

When I arrived at Columbia, John Hildebrand was teaching the cellular and molecular neuroscience course and I began teaching a complementary developmental and systems neuroscience course (always including bat echolocation) that I continue to teach to this day. I alternate with Rafa Yuste, who I recruited to Columbia during my brief stint as chair of biological sciences. In 1995, with Don Hood in psychology, I also created the neuroscience and behavior major that has had broad appeal for Columbia undergraduates.

Columbia College is distinguished by its Core curriculum: "The central intellectual mission of the Core is to provide all students with wide-ranging perspectives on significant ideas and achievements in literature, philosophy, history, music, art, and science" (Kelley, 2010). The Core originated in 1919, but science was not added to its mission until 2004. With David Helfand in astronomy and fellow science faculty members, I helped to create a new core course, Frontiers of Science (FroSci). FroSci started as a discussion in the Committee on Science Education about what students generally took to "satisfy" the science requirement, then two courses in one discipline for depth and one course in another for breadth. The problem as we saw it was that, as a general rule, only science majors had any contact with modern science. We proposed a one-semester course for all entering students, comprising both life and physical sciences, that would be taken by all first-year Columbia College students. The original idea was that faculty members would deliver one lecture, but Brian Greene pointed out that presenting a narrative really required three lectures. I was enthusiastic and so David and I went from one science department to another, promoting the idea and recruiting a small band of fellow scientists of like opinions. The inaugural Howard Hughes Medical Institute (HHMI) Professors program supported my efforts in FroSci. Today, convening yearly with my fellow, distinguished science educators and researchers (many of whom are members of the NAS) has been incredibly helpful, particularly in discovering new approaches (such as "intelligent tutors") to help level the playing field for a talented entering class with diverse preparation in science. FroSci now includes three weekly lectures from effective science communicators among the research faculty. Student skills are honed by weekly small seminars led by postdoctoral Columbia Science Fellows dedicated to teaching.

Of every hurdle that I have faced, FroSci was the most difficult. We were strongly backed by the then deans of the college (Austin Quigley and Kathryn Yatrakis). The faculty were skeptical, however, most students were hostile, and the ratings were abysmal. Strenuous efforts were initiated by a new dean and other faculty to come up with a replacement course that would be more popular. However, everyone who was interested enough to teach science to first-year students was already busy with other initiatives or teaching in FroSci. Then in 2007, David went to a new college in Canada, Quest University (eventually as its president), leaving me to hold the fort, luckily with Dr. Ivana Hughes, a chemist and gifted educator. We struggled

on, and David returned in 2015. Finally, the course emerged triumphant during the pandemic. FroSci was exceptionally well prepared for new learning styles, and thus has inched, bit by bit, year after year, into favor with Columbia students. As Churchill said (1941 at Harrow): "Never ever, ever give up."

Graduate Education

In 1983, there was no Neuroscience Department at Columbia. Neuroscience PhD trainees at the Medical School received their PhDs from physiology, with many mentors housed in the Center for Neurobiology and Behavior that Eric Kandel (see Volume 9) had set up when he moved to Columbia from NYU. Trainees at Columbia's Arts and Sciences campus received PhDs from biological sciences or psychology. I had, by then, a strong interest in developmental neurobiology and taught a graduate course on this subject with Jane Dodd and Tom Jessell. I also took an Ion Channels course from Steve Siegelbaum and John Koester. I pictured ion channels as tiny entities with innate behaviors.

Eventually, I talked John Koester into starting a new PhD program with me: NB&B. We began with a class of three: Sally Till, Sandya Viswanathan, and Jill Willdonger. Sandya had a productive career in documentary films and is now in medical practice. Sally is at the University of Edinburgh studying autism and Jill is at the University of California, San Diego. Our NB&B graduate program currently attracts 600–700 applicants annually for a typical entering class of 12 students. I continue to teach all NB&B students in their first years. After John Koester retired, I led NB&B with Carol Mason and Ken Miller, who founded our program in theory with Larry Abbott. Carol now chairs intercampus neuroscience efforts at Columbia. Ken and I were joined by Wes Grueber and Ai Yamamoto at Columbia's Medical School. Aniruddha Das (primate visual system) leads our diversity, equity, and inclusion (DEI) initiatives.

Diversity, Equity, and Inclusion

Science runs on talent. Nurturing careers—from undergraduates to faculty members—has been a central focus for me. Many women undergraduates have carried out research in our laboratory and authored research papers, beginning with Patty Hannigan at Princeton and, more recently, Ursula Kwong-Brown, now a composer (PhD, University of California, Berkeley), with whom I continue to collaborate. I fought for many years to bring more women faculty into the biological sciences. At first, the issue was advancing these recruits through tenure and then the issue was retaining them in the face of outside offers. We had much better luck recruiting senior women scientists. When Oliver Hobert moved from the medical

school to biological sciences, recruiting young women faculty members shifted into high gear. Three extraordinary new women faculty (Maria Tosches, Erin Barnhardt, and Laura Duvall) joined biological sciences just as the pandemic began. Our department and the Zuckerman Institute also had the good fortune to attract Ishmail Abdus-Saboor from Penn. Marty Chalfie in biological sciences now leads our DEI efforts. In 2020, a group of undergraduates in our lab founded the Research and Diversity (RnD) initiative to bring undergraduate scientists into conversations with researchers from underrepresented groups. NB&B graduate student have also just created a program, CAN, to coach college students from underrepresented groups with the graduate application process. Fingers crossed for all of these efforts as well.

Coda

I write this essay during the time of Covid from Baltimore, the home of my beloved second husband, Kenneth Frank. I met Kenny on Amtrak in the café car 16 years ago when he commented on my screen saver (blue-footed boobies) and asked if I had been to the Galapagos. Between us, we have four children and eight grandchildren, each one talented and beautiful. Separation from them during the pandemic has been hard. When I finally saw my grandson, he wept for the loss of a year without his friends. This spring and early summer, the 17-year cicadas emerged in Baltimore. The old oak trees were festooned with their husks. They hid under leaves from the cold rain and decorated the tires of the car. Kenny described them as "kind and curious," although not everyone agreed.

In February and March of 2020 many friends and even our young graduate students fell ill. We had no Covid tests for a long time, but we all knew. All recovered but many, many, others did not. The still-ongoing pandemic has been the most remarkable period, so far, that I have lived through; such a tricky virus. Who is most at risk, how to treat the infection and its consequences, and how to protect the world preoccupied us then and now. I virtually attended webinars across the city, the United States, and the world. We saw researchers pool resources; experienced highly collaborative science and were encouraged when its application flourished. However, we also learned that superb science is not enough to prevent illness: people, and above all policymakers in government, must believe in and act on the science.

However, in the emerging "variants" of Sars-CoV-2, we can see, in real time, how organisms evolve, including the contributions of small genetic changes to their reproduction and survival. We also have come to a greater understanding of human biology, particularly the damage that can be inflicted by our own immune system. Laboratory research, except for Covid, was paused and scientists put aside their projects to try to help out by, for

example, developing home tests for Covid that regular people can afford. Ivana Hughes and David Helfand pivoted to incorporate Covid into FroSci.

Some years ago, my last FroSci lecture on the evolution of language concluded with the "tangled bank" quote from Darwin's *Origin of Species*:

> It is interesting to contemplate an entangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent on each other in so complex a manner, have all been produced by laws acting around us. These laws, taken in the largest sense, being Growth with Reproduction; Inheritance which is almost implied by reproduction; Variability from the indirect and direct action of the external conditions of life, and from use and disuse; a Ratio of Increase so high to lead to a Struggle for Life, and as a consequence to Natural Selection, entailing Divergence of Character and the Extinction of less-improved forms. Thus, from the war of nature, from famine and death, the most exalted object which we are capable of conceiving, namely, the production of the higher animals, directly follows. There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved (Darwin, 1872).

Afterward, a student approached me to say that this view of the world seems utterly bleak to him. It does not seem bleak to me. I find this view of life wondrous. Understanding the little, red-eyed cicada, tucked underground as a larva for 17 years, feeding off the sap of trees, answering the mysterious signal to bore upwards, crawl up the trees or telephone poles, sing, mate, and then die does not take away the joy of unravelling their mystery. I do admit that periodic cicadas are not perhaps—as Andres Bendesky pointed out to me—a great model species for the lab, but they are an inspiration. The virus that now governs our lives (and deaths) is also an example of evolution. It is tempting to confer intent (such a tricky virus) but really all that is in play is selection for the ability to survive and replicate.

The evolution of neural circuits that have enabled our own species to survive this period are only one example of the "endless forms most beautiful and most wonderful" of nervous systems on our planet. We will need these and more to continue to survive the havoc we've unleashed on the planet lest it goes on cycling without us.

From Baltimore and New York City, November 2021

This memoir is dedicated to the memory of my lifelong friend, Adele LeBourgeois Alsop, who was, unbeknownst to almost all of us, bravely dying as I wrote this.

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